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Palm Tissue Culture

ABSTRACT

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Despite the economic importance of palms, little is known of palm morphogenesis and especially of means to induce their mass vegetative propagation. Limited suckering occurs among the species (for example, date palm), but numerous species cannot be vegetatively propagated at all (for example, coconut and oil palms). Palms are difficult to study because they are long-lived, genetically heterozygous, and often require unique physical environments for proper tree and fruit maturation (that is, subtropical and tropical climates). Tissue culture techniques were developed as an alternative method to grow and study desired palm clones. Detailed step-by-step procedures to propagate palms through shoot tip and embryo culture and asexual embryogenesis via callus are presented in this publication. Also, a survey utilizing 58 palm species of diverse taxonomic and geographical backgrounds was undertaken to determine the morphogenetic potential of Arecaceae in vitro. Tissue culture techniques were used to study flowering, rooting and suckering of palms in vitro. This publication is presented as an early systematic approach to study palm growth under axenic conditions.

KEYWORDS: Arecaceae, asexual embryogenesis, date palm, embryo culture, flowering, Palmae, shoot tip, suckering, tissue culture

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CONTENTS

Palm Tissue Culture

By Brent Tisserat[1]

INTRODUCTION

The agricultural importance of palms has been well documented throughout the history of the human race. Palms, being monocotyledons are restricted to axillary bud outgrowths during their juvenile life-cycle as their only means of vegetative propagation, for example, date palm, Phoenix dactylifera. Only a finite number of additional shoots can be produced per tree. Unfortunately, several important palm species even lack this means of vegetative propagation, for example, coconut palm, Cocos nucifera, and oil palm, Elaeis guineensis. Some isolated cases of dichotomous branching and tetralogical occurrences have been reported in various palms resulting in limited vegetative reproduction but it is highly unlikely these occurrences can be exploited for mass clonal propagation. Micropropagation of palms through tissue culture appears, then, to be a viable option for cloning these arborescent monocots (2, 10, 12, 15, 37, 38, 41, 42, 48, 78).

This publication is designed specifically to provide the step-by-step procedures for the tissue culture of palm species using a variety of explants and to standardize the techniques. The publication offers an initial scheme of procedures which when used will allow certain morphogenetic events to occur from various palm explants. These procedures and resultant morphogenetic events are by no means absolute. Techniques and media in the tissue culture of any plant will probably be improved for future in vitro progress whether for research or commercial exploitation.

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A detailed literature review has already been published on this subject (15, 41, 81). For the reader's interest, I have listed palm species cultured in vitro up to October 1983 in table 1. According to the literature, about 62 species from 36 genera have been cultured using various tissue culture techniques thus far, although not all the procedures were designed to produce plantlets in vitro. In some experiments, tissue culture procedures were used to test pollen viability or study morphogenetic development (14, 26, 90).

Table 1 lists the results obtained through the tissue culture of many palm species conducted in the laboratory and represents the efforts of various collaborations (see acknowledgments). Production of free-living palms has occurred from excised zygotic embryos via direct germination in 19 species, from asexual embryos in 2 species, and from cultured shoot tips in 5 species. Asexual embryos produced from callus either zygotic or somatic in explant origin have been reported in 14 species.

PROCEDURES TO TISSUE CULTURE PALMS

Palm tissue culture studies may be divided into three categories with distinct objectives of (1) clonal propagation, (2) embryo culture, and (3) growth and developmental studies. Clonal palm propagation clearly is currently drawing the most interest and research effort (37, 77). Production of clonal palms in vitro is being studied to obtain many genetically uniform trees that have merit qualities, such as high yields of fruit or disease resistance. Potentially, clonal propagation can be accomplished through either asexual embryogenesis (plantlet formation by the initiation and germination of a somatic embryo) or organogenesis (the sequential formation of roots from shoots or vice versa).

Embryo culture is the excision and germination of an isolated embryo. It may be used to produce rare incompatible crosses that would not survive development in the seed or used to increase seedling population yields through controlled germination (3, 21, 22, 23, 24, 25, 37, 46, 70, 88, 89).

Tissue culture techniques also provide promising avenues to develop an understanding of heretofore little understood aspects of palm growth, morphogenesis, and physiological processes. For example, no method is currently known to stimulate flowering or suckering in palms or to reverse adult palms to the juvenile developmental stage (48, 63, 65). Preliminary experiments have shown that flowering and suckering processes may occur in vitro, which might make controlled study of them possible.

Although the Arecaceae family is large and diverse, a general similarity appears to exist regarding the response of palm tissues to in vitro conditions. I was able to obtain callus and/or germination from excised embryos, and callus and/or leaf and root development from excised apical tips from many palm species by employing techniques and nutrient media developed initially for the date palm (table 1). The procedures used in this study follow. They should not be taken as absolute, however, as they will probably be modified through future research.

Germination of Palm Embryos

Step 1. Obtain the seed by first making two shallow longitudinal incisions into the fruit wall with a sharp knife. Clean mature seeds thoroughly with a scrub brush to remove fruit debris clinging to them as debris left on the seeds increases chances of contamination from seeds. Soak clean seeds in tap water for 24 to 48 hours to hydrate the embryo and endosperm and soften the seedcoat for easier opening of the seed. Some palm seeds such as oil palm, however, have such a hard seedcoat that seeds must be cracked open in a vise. To do this, wrap seeds in a towel, insert towel into the vise, and crack seed open by slowly applying pressure. Remove the embryo and treat as seeds in the following step.

Step 2. Immerse the extracted seeds in a 2.6 percent sodium hypochlorite solution (containing 1 drop Tween-20 emulsifier per 100 mL solution) for about 15 to 30 min. Drain off the disinfectant solution and transfer seeds to a 15 x 150 mm petri dish. Perform all subsequent operations in a laminar airflow hood using aseptic techniques.

Step 3. Select individual seeds to extract the embryos from the petri dish using a bayonet forceps 19 cm long. Hold the seed firmly between thumb and index finger, cut the seed lengthwise at the opposite ends of the furrow (if one is present) with an anvil hand cutter (presoaked in 95 percent ethanol and flame treated). The halved seed will open exposing the embryo without damaging it.

Step 4. Remove the exposed embryo using a surgeon's scalpel fitted with a No. 11 surgical blade either by (1) piercing the haustorium end of the embryo (that is, the end most embedded within the seed) or

(2) lift the embryo out of the endosperm-embryo cavity with the blunt side of the scalpel blade to avoid damaging the embryo.

Step 5. Place the excised embryo carefully lengthwise on the surface of the agar medium. Avoid any further embryo damage or immersing the embryo into the agar medium.

Step 6. Incubate embryos on nutrient medium described in table 2 (plantlet-germination medium) under a 16-hr photoperiod illuminated with 50 fc light intensity provided by Gro-lux fluorescent lights at 28 °C in an environmental chamber. Embryos will begin to enlarge and exhibit germination within 1 to 2 weeks after planting. Plantlets will usually grow to 2 to 6 cm long after 8 weeks in culture and will possess a primary root system and first foliar leaf (figs. 4, 5, and 6).

Step 7. Reposition the embryo during this first culture transfer so that the root portion is embedded in the agar medium and the shoot and leaves grow upwards.

Step 8. Reculture the seedling on adventitious rooting medium with the primary root trimmed to 1 to 2 cm long to enhance adventitious root formation (table 2 and figs. 7 and 8). Continue the reculturing procedure every 8 weeks for 2 or 3 culture passages until seedlings reach a length of 10 cm with 2 to 3 leaves and have a well-developed adventitious root system.

Step 9. Transfer plantlets to free-living conditions as follows: Remove plantlets carefully from agar medium without damaging the root systems and soak in distilled water for 15 min to avoid dehydration and to remove excess media that may be adhering to them. Rinse plantlets three times with distilled water, spray them with 0.5 percent benolate containing active ingredient benomyl (DuPont, Wilmington, DE) fungicide solution and transfer plantlets to soil medium, which consists of peat moss and vermiculite in a 1:1 v/v ratio. Pot plantlets in either 7.6-cm diameter plastic or jiffy peat containers and enclose within a transparent tent composed of two interlocking clear polystyrene tumblers.

Step 10. Spray foliage weekly with 0.5 percent benolate to minimize fungal growth. Water pots every other day with distilled water and once a week with one-fourth strength Hoagland's solution during the first 2 months of plantlet development. Incubate plants initially in an environmentally controlled chamber under 800 fc light intensity, 16-hr photoperiod at 28 °C for 2 weeks, then transfer to a shaded greenhouse. Gradually acclimate plantlets to the greenhouse humidity conditions by punching holes in the plastic cover. After 2 mo remove covers and treat the plant thereafter as a palm seedling (fig. 9).

Plantlets From Embryo Callus

1. Perform steps described for the excision and planting of zygotic embryos as described in steps 1 to 6 of previous section "Germination of Palm Embryos."

2. Incubate embryos on callus production medium (table 2).

3. Reculture explants at 8-week intervals. Nodular yellow-brown callus initiation becomes evident after 2 to 3 culture passages (figs. 4 and 10).

4. Eventually the original embryo structure becomes obliterated through the production of white-yellow friable, and nodular callus. Subculture 1-cm^2 pieces to plantlet germination medium (table 2). Asexual embryos and green plantlets usually will become apparent within 2 to 4 weeks in culture (fig. 11).

5. Follow steps 7 to 10 in section "Germination of Palm Embryos" procedure to obtain free-living plantlets.

Plantlets From Shoot Tip and Lateral Bud Callus

1. Remove offshoots from the parent tree by severing the vascular connection with either a sledge hammer and chisel (fig. 1) or a chain saw (fig. 2). Shoot tips can be extracted from adult trees by first making a circumrotated shallow cut with a chain saw near the tip and removing the outward debris (fig. 2). The shoot tip region can be found by peeling off leaves while trees are still in the field. Dissect offshoots or trees using a hatchet and serrated knife. Remove leaves acropetally, exposing lateral buds at the axil of each leaf (fig. 3). Shoot tips are obtained after peeling off all mature leaves. Lateral buds in various stages of differentiation occur within the same shoot (fig. 3). Store buds and tips in a cold antioxidant solution (150 mg/L citric acid and 100 mg/L ascorbic acid). Keep explants in refrigerator at 0 °C until conducting the surface sterilization procedure (preferably within 24 to 48 hrs).

2. Trim away the outermost leaves of buds and tips to obtain explants that are about 0.5 cm^2.

3. Sterilize explants by wrapping them in cheesecloth to prevent loss in handling procedures and place in a 25- X 150-mm culture tube. Sterilize in 2.6 percent sodium hypochlorite solution (containing 1 drop of Tween-20 per 100 mL solution) for 15 min. Agitating the culture tube periodically to dislodge air bubbles from tissues. Pour off bleach solution and rinse three times with sterile water. Remove explant package and transfer aseptically to the sterile petri dish (15- X 150-mm dia.).

4. Remove additional leaves one at a time from shoot tip and bud explants to obtain a 1- to 3-mm^2 culture. An additional 10-sec dip in the bleach solution of this explant before planting may further reduce contamination.

5. Plant explant on the surface of "callus production medium" described in table 2. Orient apical end aerially upwards.

6. Follow steps 3 to 5 in the "Plantlets From Embryo Callus" procedures to produce free-living palms via callus (fig. 14). Embryogenetic callus derived from shoot tip explants behaves very similarly to that obtained from zygotic embryos (figs. 12, 13, and 14).

Palms Via Shoot Tip Proliferation and Rooting

1. Repeat procedures for excision and planting of shoot tips and lateral bud explants as described in steps 1 to 4 in previous section "Plantlets From Shoot Tip and Lateral Bud Callus."

2. Plant explants on the surface of shoot tip medium or on adventitious rooting medium described in table 2.

3. Cultures will initiate leaves and enlarge considerably within the next 4 to 6 weeks in culture. Reculture explant to fresh media at the end of the 8-week culture passage (figs. 15, 16, and 17).

4. Reculture tips to shoot-proliferation medium to induce axillary proliferations (figs. 16 and 17).

5. Follow steps 8 to 10 in the "Germination of Palm Embryos" procedures to root buds and tips and obtain free-living palms (fig. 17).

CASE HISTORIES

General Remarks

The following cases of palm tissue and organ growth are an accumulation of several years of research endeavors in which transfer of knowledge initially gained by the culture of the date palm was used on other palm species (79, 80, 81, 82, 83, 91) (table 3). No attempt was made to develop specific media and techniques for each palm species studied because palm explant anatomy, structure, and cultural behavior were often quite similar regardless of the species studied.

Aside from the procedures offered here, the following discussion might be useful to other palm researchers. Initially, palm growth in vitro was much slower than that for herbaceous plant species. Several investigators reported that palm callus production is long term (15, 79, 84). Cultured embryo and tip explants will produce some callus after 4 to 8 weeks on media containing hormones (fig. 4). This callus is usually the hard nodular enlargement of the explant itself. More friable callus is produced through later recultures. The visible production of asexual plantlets was usually not apparent until nearly 4 to 6 months later (figs. 11, 13, and 14).

Recultured palm callus, regardless of the species, was composed of two distinct coexisting types of tissues--friable and nodular components (15, 84). The friable callus may be embryogenic (84) and is sometimes genetically unstable (15, 76). The nodular callus bodies are often the source of asexual embryos or are themselves asexual embryos (15, 84). In our studies, root formation from callus often occurs from nonembryogenic callus lines (81, 92). A great deal of genetic variation in the callus population apparently exists (15, 84). Choo, Yew, and Corley (15) reported that generally fast-growing callus lines in oil palm were usually not embryogenic although there were exceptions (60). In date palm, embryogenic callus appears to develop from initial slow-growing callus, following 4 to 6 months in culture. Once this callus is isolated, it may be then rapidly multiplied as a callus/embryo mass. Generally, each palm explant established grew as a clone in vitro with unique growth characteristics. Similarly, explants obtained from shoots of the same clones exhibited this individualism. Some clonal characteristics established in vitro included callus growth rate, callus composition, and embryogenic or organogenic ability. I believe that the inherent explant character rather than medium composition is the determining factor in obtaining growth or organogenesis from palm tissue cultures.

Best success in establishing embryogenic callus has been obtained from cultured apical tips, lateral buds, and embryos rather than from more physiological mature explants obtained from leaves, stem, roots, and inflorescences. However, the tree must often be sacrificed when tips are used as explant sources. Generally, about 60 to 80 percent of the palm shoot tips and 30 to 100 percent of the cultured embryos can be successfully established in vitro. Other investigators, working with oil palm, have been able to obtain embryogenic callus from inflorescences, leaves, petioles, and roots (15). These explants, however, were only established at low rates of survival in culture (that is, 5 to 10 percent). Immature zygotic embryos should be better explant sources in establishing morphogenetically active callus than mature embryos obtained from ripe fruit, although both have been successfully used to produce embryogenic callus (45, 63, 68, 79).

Various abnormalities in seedling and plantlet development have occurred in vitro such as plantlets becoming disoriented in development that results in the shoot and root systems becoming intertwined (fig. 21). Other odd growth patterns observed are retarded germination, abnormal leaf curling, and leaf swelling. Whether these abnormalities,

which occur in about 5 to 10 percent of the plantlets, are genetic or environmental is unknown.

Growth Responses of Excised Embryos

A study was made to determine the morphogenetic growth responses of palm species using excised embryos as the explant source. Excised embryos were used because they are inherently meristematic and contain the genetic composition of the adult trees. Clonal palm material is not easily obtained in the temperate regions of the United States. Table 3 lists the members of the Arecaceae that were studied in vitro using excised zygotic embryo explants isolated from mature seeds. Seed source, seed and embryo size, habit, and distribution are included to provide a brief sketch of the species background. To determine their morphogenetic behavior in vitro, mature zygotic embryos were planted on basal nutrient medium containing charcoal with and without hormones. Observed growth responses over a 24-wk culture period are presented in table 4.

A wide variety of growth responses from excised embryos occurred among and within the species tested utilizing the medium and techniques described in the procedure sections (table 4). On the medium with charcoal only, embryos either germinated completely to form plants that could be eventually transferred to free-living conditions or germinated partially (that is, cotyledon and, sometimes, root elongation). Within the first week of culture, embryos enlarged notably. Usually cotyledon elongation and greening of the embryo occurred in the second and third week of culture. However, germination rates of embryos within the same species were not synchronized (figs. 5 and 8).

The emergence of the primary root and first foliar cotyledon usually occurred during the third through eighth week of culture (figs. 4 to 8). A few embryos did not completely germinate until recultured to fresh medium. Plantlets exhibited geotrophic responses with the shoot end erect in the air and the root end embedded in the nutrient medium. The cotyledon haustorium was nonfunctional and much smaller for most cultured embryos regardless of the species compared with that found in germinating seeds (fig. 5).

Germination rates varied from 10 (*Caryota mitis*) to 100 percent (*Phoenix dactylifera*). Of the 52 cultured species, 25 of them exhibited a complete germination response while 20 others exhibited only partial germination (that is, cotyledonary elongation) (table 4). The remaining seven species did not grow at all. Much variation in total number of leaves, seedling length, and root development occurred among and within the cultured palm species (table 4). Adventitious root formation was achieved from germinated embryos through their reculture to nutrient medium without charcoal and supplemented with 0.1 mg/L NAA.

When excised zygotic embryos were cultured on nutrient medium containing hormones, germination was usually suppressed and callus initiation occurred (table 4). However, various morphogenetic responses were also observed from cultured embryos on this medium including cotyledonary elongation, embryo germination, adventitious rooting, callus formation and asexual embryo production via callus. Ten species exhibited cotyledon elongation only from culturing embryos in this medium (table 4). Germination resulting in the formation of plantlets occurred in 10 species on this medium (for example, *Elaeis guineensis*). Callus production from cultured embryos occurred in 38 of the 52 species cultured. Initially, the texture of the callus formation was hard; however, following successive recultures, friable and nodular callus was produced.

The frequency of callus formation varied from 10 (*Dictyocaryum platysepalum*) to 100 percent (*Elaeis guineensis*). Excised embryos often enlarged and callused within the first 5 weeks of culture. Generally, those embryos that did not form callus during the first 8-wk culture

period usually died. Following 24 weeks in culture, excised embryos from several palm species produced healthy callus that sometimes gave rise to asexual embryos (figs. 10 and 11). In 10 species cultured (for example, Brahea armata, B. dulcis, Erythea edulis, Livistona decipens, Phoenix canariensis, P. dactylifera, P. pusilla, P. sylvestris, Prestoea sp., and Sabal minor), asexual embryos were produced from callus (table 4).

Interestingly, some species died on the medium without hormones but exhibited some growth responses on medium with hormones (for example Aiphanes caryotifolia, Cocos nucifera, C. pulposa, Elaeis quineensis, and Sabal minor, while the opposite occurred for Pinanga copelandii, Sabal palmetto, and Washingtonia robusta) (table 4).

Shoot Tip Growth In Vitro

Depending on the nutrient medium used, alternative morphogenetic responses could be obtained from cultured palm shoot tips. Tips cultured on nutrient medium supplemented with 0.1 mg/L NAA or on medium containing 0.3 percent charcoal and 10 mg/L NAA often produced complete plantlets through leaf and shoot organogenesis (figs. 15 to 17). On basal nutrient medium containing 0.3 percent charcoal, 100 mg/L 2,4-D and 3 mg/L 2iP, shoot tip growth was inhibited and callus was produced from the leaves after 4 to 6 weeks in culture (fig. 12). This callus was yellow brown and nodular. On subculture to fresh nutrient medium, more rapidly growing friable and nodular callus pieces were produced from this nodular callus (fig. 12). After 4 to 6 months in culture, both friable and nodular callus occurred from the same cultures (fig. 12). Of the 15 palm species and cultivars shoot tips cultured in vitro, 7 exhibited asexual embryos and plantlet formation following 32 weeks in culture. These asexual embryos were produced from only selected parts of the callus. Initially, few asexual embryos were produced but through selection and subculturing of the embryogenic parts of the callus many asexual embryos could be produced per culture.

In date palm, up to 500 asexual embryos were produced per culture every 8 weeks from about 0.5 g of callus/embryo piece (83). The mode of asexual embryogenesis from callus derived from various palm species resembled that observed in date palm (84). Embryogenetic callus contained embryos in various stages of development that eventually germinated to produce a plantlet through a sequence of development similar to that found in true embryo development occurring in the seed (figs. 12 to 14).

When shoot tips were cultured on nutrient medium containing lower auxin concentrations, shoot and root organogenesis readily occurred resulting in the formation of a complete plantlet (figs. 15 to 17). In the first 4 weeks of culture, tips enlarged several fold and initiated several leaves. After 6 to 10 weeks, the shoot reached sufficient size to develop an adventitious root system (fig. 17). After several recultures to enhance shoot vigor and to stimulate a well-formed adventitious root system, shoots could be transferred successfully to free-living conditions (fig. 17, table 5). Attempts to initiate lateral bud outgrowths through inclusion of cytokinins in the medium were only successful in date palm and Metroxylon sp. In date palm, only a few additional shoots (1 to 6) were produced per shoot tip after 8 weeks in culture (figs. 16 and 17). Additional shoot production was noted in about 10 to 20 percent of the shoot tip cultures (fig. 17). Other investigators have reported production of additional shoots from tip cultures of date palm but not the frequency of the occurrence (49, 70). Reculturing shoots in a medium containing 10 mg/L 2iP and 0.1 mg/L NAA generally increased the number of axillary shoot outgrowths produced per culture. Up to 20 shoots have been obtained from a single shoot tip using this method after 6 months of culturing (figs. 17 and 18). One culture of Metroxylon sp. produced an additional shoot after 16 weeks in culture.

MORPHOGENETIC STUDIES IN VITRO WITH PALMS

The mechanism of lateral bud initiation in palms is not understood and has eluded control (48). There is increasing evidence that tissue culture techniques may become instrumental to study lateral bud initiation and development in palms versus traditional field and greenhouse studies. Both flowering and suckering have been achieved from cultured date palm zygotic embryos, asexually derived plantlets, and shoot tips (figs. 18, 19 and 20). Further, tissue-cultured palms are smaller than their in vivo counterparts and thus it is easier to handle large experimental populations in small areas.

Cultured date palms may reproduce several life-cycle events within a few months timespan, while in nature these same events require years to occur. Tissue culture studies of flowering and suckering involve acceleration of the timeframe in which these events occur: in nature, date palms usually begin to produce suckers in their late juvenile stage, about 3- to 7-year-olds. Flowering formation and the adult-life cycle stage may occur at 5 to 7 years. In vitro, however, both flowering and suckering occurred from cultures that were as young as 6- to 12-wks old.

Production of lateral bud outgrowths from palm plantlets derived from zygotic embryos (5, 34, 37), asexual embryos (83), and shoot tips (49, 69) cultured in vitro has been reported (fig. 18). These axillary branches may be either vegetative or reproductive. All described instances of production of lateral bud outgrowths have been reported in the date palm except for a single case of branching in coconut (34).

Vegetative lateral bud outgrowths have occurred in less than 10 percent of the cultured zygotic embryos and 10 to 20 percent of the shoot tip cultures. Asexual plantlets derived from callus produced a much higher frequency of lateral bud outgrowths, about 40 percent. Vegetative outgrowths originated from lateral buds located at the leaf axil (fig. 18). The number of lateral buds produced from a single plantlet is not influenced by the media composition, although cytokinins enhanced shoot development somewhat. The genetic nature of plantlets profoundly influence shoot development in vitro as it does in vivo, for example 'Medjool' date palm asexual plantlets produced more shoots than other asexual plantlet clones of date varieties.

Production of flowers from cultured palm explants was a relatively rare event and occurred in only 2 to 5 percent of the cultures (figs. 19 and 20). Date palm plantlets that produced inflorescences exhibit their typical dioecious nature (that is, male and female flower on separate plants). Male flower buds normally produce three sepals, three petals, six bilobed anthers with microsporangia containing typical monosulcate elliptical pollen. Male flowers also contain three pseudocarpels (vestigial carpels) (fig. 20). Pseudocarpels are located in the flower's center and may expand into parthenocarpic fruit occasionally (26). This vestigial trait is retained from ancestral palms that possessed perfect flowers. Pistillate flowers produced in vitro resemble their in vivo counterparts and contain an ovule. Flower maturation was basipetal.

Understanding the mechanism of lateral bud initiation and control in vitro may be transferred to palms grown in the field and greenhouse. Potentially, future breeding and physiological studies can be performed year round in culture vessels.

CONCLUSIONS

Problems that hinder the study of palms are their long-lived nature, growth habit, and peculiar growth habitats. Suckering and flowering phenomena frequently do not occur until the third through seventh year of development. Most palms have tropical or semitropical habitats that often prevents their critical study in temperate climates. Size of adult palms in itself, presents a problem in experimental work. There is no known procedure to control lateral bud initiation thus far (48). Nor are methods available that can accelerate vegetative lateral bud outgrowths or reverse the adult to the juvenile life cycle. Outgrowth of flower and vegetative buds were obtained from cultured embryos, shoots, and asexual plantlets. Only three species--*Cocos nucifera*, *Metroxylon* sp., and *Phoenix dactylifera* (34)--produced lateral bud outgrowths in vitro.

The mechanism for their production was only preliminarily explored. Probably, the major point concerning their occurrence is that they are produced at all. Several major life cycle events of some palms can now be performed through tissue-culture techniques. This technique, however, needs to be studied for use on other species.

Plant tissue culture was studied as a technique that could be used to potentially mass produce desirable palms. Studies on date and oil palm tissue culture are more developed than for other palms because (1) they have been the focus plant in several intense research programs (for example, date (2, 5, 66, 68) and oil palm (16, 17, 18, 42, 54) and (2) both date and oil palm meristematic tissues appear to be highly totipotent. Coconut palm although continually studied over the last few decades still has yet to yield easily produced embryogenic callus (7, 9, 33, 41). Several palm species were cultured in this publication using either zygotic or somatic explant source material with the in vitro techniques developed for date palm.

Generally, direct transfer of date palm techniques to other palms can be performed to obtain plantlets from germinated excised embryos, tips, or callus. Those palms that grew poorly using the described techniques are candidates for more intensive study. Extremely small explant populations were used in this study; however, the poor results could reflect artifactual effects.

Tissue culture techniques were applied to 62 species representing 36 genera in the *Arecaceae* with varying degrees of success. Initiation of callus from embryo and shoot-tip explants should be considered only preliminarily helpful in obtaining plantlets via callus.

As restated in a previous review (81), meaningful research directions in this field should be directed at (1) determining the genetic stability of plantlets produced from tissue culture, (2) elucidating the mechanism of lateral bud differentiation on demand, and (3) maximizing plantlet production with minimum labor requirements. Endeavors into some of these projects now lie outside the aims of publicly funded research and become the responsibility of commercial enterprises.

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Table 1.
Summary of reports dealing with tissue culture
in the Arecaceae

Plant	Explant	Growth response	Reference
Aiphanes carotifolia (H.B.K.) H. Wendl.	Zygotic embryo	Cotyledon elongation	Author's data[1]
Archontophoenix alexandrae Wendl.	do.	Callus or germination[2]	Do.
cunninghamiana (H. Wendl.) Wendl. & Druce	Apical tip	Callus	Do.
	Zygotic embryo	Callus or germination	Do.
Arecastrum romanazoffianum (Cham.) Becc.	do.	Germination[2]	Do.
Arenga mindorensis Becc.	do.	Cotyledon elongation	Do.
Brahea armata (Mart.) Wats.	do.	Callus/asexual embryos or germination[2]	Do.
dulcis (H.B.K.) Mart.	do.	do.	Do.
Butia capitata (Mart.) L.	do.	Callus or germination[2]	Do.
	do.	Germination	72
Caryota mitis Lour.	do.	do.	Author's data
urens L.	do.	do.	88
Chamaedorea costaricana Oerst.	do.	Callus/asexual embryos	67,68
excelsa	Apical tip	Callus	Author's data
glaucifolia Wendl.	Zygotic embryo	Callus or germination[2]	Do.
humilis (Liebm.) Burret	do.	Cotyledon elongation	Do.
radicalis Mart.	do.	do.	Do.
Chelyocarpus thindera Wendl.	do.	Callus or germination[2]	Do.

Table 1--Continued
Summary of reports dealing with tissue culture
in the Arecaceae

Plant	Explant	Growth response	Reference
Cocos			
nucifera L.	Apical tip	Shoot and root differentiation[2]	9
	Established callus	Protoplasts	10
	Inflorescence	Callus/roots	9,10,27,28,29
	do.	Callus/shootlets	9,10,27,28,29
	do.	Protoplasts/callus regeneration	36,41
	Leaf/petiole	Callus/roots	10,27,28
	Root	Lateral root initiation	10,35
	Stem	Callus/roots	7,27,28,29
	Zygotic embryo	do.	33
	do.	Callus/asexual embryos	25,33
	do.	Germination	3,8,20,21,22,23,24,41,46,70,88
	do.	Germination/branching	34
pulposa Becc.	do.	Callus	Author's data
Corypha elata L.	do.	Callus or germination[2]	Do.
Chrysalidocarpus lutescens Wendl.	do.	do.	Do.
Dictyocaryum platysepalum	do.	Callus	Do.
Elaeis quineensis Jacq.	Apical tip	Callus/asexual embryos[2]	15,16,17,18,19,39,40,62,76
	do.	Callus	47
	do.	Leaf differentiation	47
	do.	Leaf differentiation and root initiation	76
	Inflorescence	Flower development	1,47
	Leaf	Callus/asexual embryos	2,42,61,62
	Pollen	Germination	14
	Root	Callus/roots/pneumatodes	43,44
	do.	Callus/asexual embryos	17,18,19,39,40,76
	do.	Root elongation	43,44
	Zygotic embryo	Callus	47
	do.	Callus/asexual embryos[2]	40,54,57,59,75,76

Table 1--Continued
Summary of reports dealing with tissue culture
in the Arecaceae

Plant	Explant	Growth response	Reference
Elaeis quineensis Jacq.	do.	Germination[2]	11,13,47,50, 51,52,53,55, 56,57,58,59, 60,61,62
Erythea edulis (H. Wendl. ex Wats.) S. Wats.	Apical tip	Leaf and root initiation or callus/asexual embryos	Author's data
	Zygotic embryo	Callus/or asexual embryos or germination[2]	Do.
Heterospahte elata Scheff.	do.	Callus or germination[2]	Do.
Howeia forsteriana Becc.	do.	Callus/asexual embryos	67,68
Hydriastele sp.	do.	Callus or germination[2]	Author's Data
Licuala spinosa Thumb.	do.	Callus	Do.
Livistona decipiens Becc.	do.	Callus/asexual embryos	Do.
merrillii Becc.	do.	Callus	Do.
saribus (Lour.) Merr. ex. A. Cheval.	do.	Callus or germination[2]	Do.
Mascarena lagenicaulis L.	do.	do.	89
vershaffeltii L.	do.	do.	89
Normanbya normanbyi Bailey	do.	Callus	Author's data
Onocosperma horridum Scheff.	do.	Germination[2]	Do.
Opsiandra maya Cook.	do.	Callus/germination[2]	Do.

Table 1--Continued
Summary of reports dealing with tissue culture
in the Arecaceae

Plant	Explant	Growth response	Reference
Phoenix canariensis Hort. ex. Chabaud.	Apical tip	Callus or leaf and root initiation[2]	Do.
	Zygotic embryo	Callus/asexual embryos	Do.
canariensis x dactylifera	Apical tip	Callus	Do.
dactylifera L.	Anther	Asexual embryogenesis	12
	do.	Failure to grow	4
	Apical tip	Leaf and root initiation/ axillary branching[2]	49,69
	Apical tip and lateral buds	Leaf differentiation	30,48,64,65, 71,78,79,80, 85,90
	do.	Leaf differentiation and root production[2]	48,49,64,65, 66,69,79,80, 81,85,90,91
	do.	Callus	48,64,65,66, 89
	do.	Callus/asexual embryos	31,32,79,80, 81,82,83,84, 85,86,87,91
	do.	Inflorescence initiation	Author's data
Phoenix -- dactylifera L.	Asexual plantlets	Axillary branching[2]	83
	Flower buds and inflorescence	Anthesis/flower differentiation	26,81,84
	Fruit mesocarp	Callus	73
	Inflorescence	Callus/asexual embryos	67,68,79
	Leaf	Callus/roots	91
	Meristele	Callus	79,83,91
	Petiole	Callus/roots	28,29,73,91
	Polyembryonic embryos	Callus/asexual embryos	63
	Root	Plantlet initiation	74
	do.	Callus	12,91
	Seed	Callus/asexual embryos	5
	Zygotic embryo	do.	5,6,45,67,68, 79,80,81,84
	do.	Inflorescence formation	5,41

Table 1--Continued
Summary of reports dealing with tissue culture
in the Arecaceae

Plant	Explant	Growth response	Reference
Phoenix--			
dactylifera L.	do.	Germination	48,64,65,66, 71,72,73,79, 80,81
hamefarra formasana	do.	Callus or germination	Author's data
humilis Royle	Apical tip	Callus	Do.
pusilla Gaertn.	Zygotic embryo	Callus/asexual embryos	Do.
reclinata Jacq.	Apical tip	Callus	Do.
	Zygotic embryo	do.	Do.
roebelenii O'Brien	Apical tip	Callus/asexual embryos	Do.
rupicola Anders.	Zygotic embryo	Callus or germination	Do.
sylvestris (L.) Roxb.	Apical tip	Callus/asexual embryos	Do.
	do.	Leaf differentiation and root formation	Do.
	Zygotic embryo	Callus/asexual embryos	Do.
Pigafetta filaris Harkes	do.	Callus or germination[2]	Do.
Pinanga copelandii Becc.	do.	Germination[2]	Do.
Prestoea sp.	do.	Callus/asexual embryos	Do.
Pritchardia kaalae Rock	do.	Germination	37
Ptychoperma microcarpum	do.	do.	Author's data
Rhopalostylis sapida H. Wendl. and Druce	do.	Callus or germination	Do.
Sabal domingensis Becc.	do.	Cotyledon elongation	Do.
etonia Blatter	do.	Callus or germination	Do.
minor (Jacq.) Pers.	Apical tip	Leaf differentiation	Do.
	Zygotic embryo	Callus/asexual embryos	Do.
palmetto	do.	Cotyledon elongation	Do.

Table 1--Continued
Summary of reports dealing with tissue culture
in the Arecaceae

Plant	Explant	Growth response	Reference
Thrinax radiata Lodd. ex Desf.	do.	Callus or germination[2]	Do.
Trachycarpus fortunei (Hock.) H. Wendl.	do.	Germination[2]	Do.
Veitchia joannis H.	do.	do.	37
Veitchia merrillii (Becc.) Moor.	do.	Callus or germination	Author's data
Washingtonia filifera (Linden ex Andre) H. Wendl.	Apical tip	Callus	Author's data
	do.	Leaf differentiation and root formation[2]	Do.
	Zygotic embryo	Callus or germination[2]	Do.
Washingtonia robusta Wendl.	Apical tip	Leaf differentiation and root formation[2]	Do.
	do.	Callus	Do.
	Zygotic embryo	Germination[2]	Do.

[1]Author's data were obtained by the culture of excised zygotic embryos on a modified Murashige and Skoog medium with 0.3 percent charcoal for a germination or cotyledon elongation response, or on medium with 100 mg/L 2,4-D and 3 mg/L 2iP to produce callus and sometimes asexual embryos. Apical tips were cultured on media supplemented with 10 mg/L NAA to promote leaf and root development or on media with 100 mg/L 2,4-D and 3 mg/L 2iP to promote callus and sometimes asexual embryo formation.

[2]Denotes successful formation of plantlets that have been transplanted to soil.

Table 2.
Composition of palm media

Components	Types of media (mg/L)				
	Callus production	Plantlet germination	Shoot tip	Shoot proliferation	Adventitious rooting
Inorganic salts					
Murashige and Skoog formulation (81).	+[1]	+	+	+	+
Carbohydrate source					
Sucrose	30,000	30,000	30,000	30,000	30,000
Vitamin sources					
Meso-Inositol dihydrate	100	100	100	100	100
Thiamine•HCl	0.4	0.4	0.4	0.4	0.4
Complex addenda					
Phytagar	8,000	8,000	8,000		8,000
Charcoal, activated neutralized.	3,000	3,000	3,000		
Phytohormone					
2,4-dichlorophenoxy-acetic acid (2,4-D).	100		10		
N-(Δ^2-isopentyl) adenine (2iP).	3			10	
α-naphthaleneacetic acid (NAA).				0.1	0.1

[1]Inorganic salt formulation was added at the same concentration to all media.

Table 3.
List of palm members used in tissue-culture study

Name	Seed or plant source[1]	Size (cm)[2] Seed	Size (cm)[2] Embryo	Habit	Distribution
Aiphanes caryotifolia (H.B.K.) H. Wendl.	APS	1.7	0.18 : 0.08	Spiny solitary trunk; variable stature; monoecious; pinnate leaf.	Tropical South America.
Archontophoenix alexandrae Wendl.	APS	1.0 : .9	.1 : .05	Solitary slender trunk; tall stature; monoecious; pinnate leaf.	Australia.
cunninghamiana (H. Wendl.) Wendl. & Druce	APS	1.2	.13 : .07	Solitary trunk; tall stature; monoecious; pinnate leaf.	Tropical Australia.
Arecastrum romanazoffianum (Cham.) Becc.	APS	.9 : .4	.11 : .05	Solitary trunk; medium stature; monoecious; pinnate leaf.	Tropical South America.
Arenga mindorensis Becc.	APS	2.4 : 1.5	.40 : .23	Solitary trunk; medium stature; monoecious; monocarpic; pinnate leaf.	Tropical Southeast Asia.
Brahea armata (Mart.) Wats.	APS	1.9 : .8	.35 : .18	Solitary trunk; tall stature; hermaphrodite; palmate leaf.	Subtropical Central America (Mexico).
dulcis (H.B.K.) Mart.	APS	1.1	.10 : .06	Solitary trunk; medium stature; hermaphrodite; palmate leaf.	Do.

Table 3--Continued
List of palm members used in tissue-culture study

Name	Seed or plant source[1]	Size (cm)[2] Seed	Size (cm)[2] Embryo	Habit	Distribution
Butia capitata (Mart.) L.	LASCA	1.8 : .9	.20 : .09	Solitary trunk; variable stature; monoecious; pinnate leaf.	Subtropical South America.
Caryota mitis Lour.	BGS	1.23	.44 : .24	Multiple trunks; medium stature; monoecious; bipinnate leaf.	India & Malaya.
Chamaedorea excelsa	LN			Multiple trunks; medium stature; dioecious; pinnate leaf.	Central and South America.
glaucifolia Wendl.	APS	.5	.1 : .05	Solitary trunk; tall stature; dioecious; pinnate leaf.	Do.
humilis (Liebm.) Burret.	APS	.8	.18 : .10	--	--
radicalis Becc.	APS	1.4	.17 : .08	Multiple trunks; short stature; dioecious; pinnate leaf.	--
sp.	APS	.5	.10 : .05	--	
Chelyocarpus thindera Wendl.	LASCA	.5	.12 : .07	Solitary ringed trunk; tall stature; palmate leaf.	Tropical Central America.
Cocos nucifera	LM			Solitary trunk; tall stature; monoecious; pinnate leaf.	Tropical Asia and Pacific region.
pulposa Becc.	APS	2.4 : 1.6	.3 : .1	--	--

Chrysalidocarpus lutescens Wendl.	APS	1.5 : .7	.2 : .19	Multiple trunks; tall stature; dioecious; pinnate leaf.	Madagascar.
Corypha elata L.	APS	1.8	.15 : .06	Solitary trunk; tall stature; monoecious; palmate leaf.	Tropical Southeast Asia.
Dictyocaryum platysepalum Burret.	APS	2.9	.57 : .29	Solitary trunk; tall stature; pinnate leaf.	South America (Andes Mountains)
Elaeis quineensis Jacq.	UFC	2.6 : 2.2	.27 : .1	Solitary trunk; medium stature; monoecious; pinnate leaf.	Tropical Africa, Central America, and Southeast Asia.
Erythea edulis (H. Wendl. ex Wats.) S. Wats.	LASCA	1.5	.35 : .17	Solitary thick trunk; tall stature; hermaphrodite; palmate leaf.	Subtropical Central America (Guadalupe Islands).
Heterospathe elata Scheff.	APS	.7	.15 : .07	Solitary slender trunk; tall stature; monoecious; pinnate leaf.	Philippines and Tropical Pacific Islands.
Hydriastele sp.	APS	.95	1.66 : .1	Solitary trunk; tall stature; monoecious; pinnate leaf.	--
Howia forsteriana Becc.	APS	1.2 : .5	.5 : .15	Solitary thick trunk; tall stature; monoecious; pinnate leaf.	Subtropical New Zealand.
Licuala spinosa Thumb.	APS	.43 : .3	.1 : .1	Multiple trunks; medium stature; hermaphrodited; palmate leaf.	New Britian Islands.

Table 3--Continued
List of palm members used in tissue-culture study

Name	Seed or plant source[1]	Size (cm)[2] Seed	Size (cm)[2] Embryo	Habit	Distribution
Livistona					
decipiens Becc.	LASCA	1.2	.2 : .14	Solitary slender trunk; variable stature; monoecious; palmate leaf.	Subtropical Australia.
merrillii Becc.	APS	1.6	.3 : .14	do.	Tropical Philippines.
robinsoniana Mart.	BGS	1.9	.2 : .19	do.	Do.
saribus (Lour.) Merr. ex A. Cheval.	APS	1.2	.35 : .18	Solitary slender trunk; tall stature; hermaphrodite; pinnate leaf.	Tropical Philippines.
Normanbya					
normanbyi Bailey	APS	5.5 : 3.2	.75 : .3	--	--
Onocosperma					
horridum Scheff.	BGS	1.44	.37 : .1	Solitary trunk; tall stature; monoecious; pinnate leaf.	Tropical India.
Opsiandra					
maya Cook.	APS	1.1 : .6	.23 : .12	Solitary trunk; tall stature; dioecious; pinnate leaf.	Tropical Central America.
Phoenix					
canariensis Hort. ex Chabaud.	APS	1.7 : .6	.18 : .09	Solitary thick trunk; tall stature; dioecious; pinnate leaf.	Subtropical Canary Islands.
canariensis x dactylifera	USDCS			--	--
dactylifera L.				Multiple trunk; variable stature; dioecious; pinnate leaf.	Subtropical North Africa and Western Asia.

cv. Barhee	USDCS	1.7 : .6	.2 : .1		
cv. Deglet Noor	USDCS	2.4 : .8	.21 : .1		
cv. Halawy	USDCS				
cv. Khalasa	BLDG				
cv. Medjool	USDCS	2.36 : 1.02	.19 : .1		
cv. Sayer	USDCS	2.4 : .8	.21 : .1		
cv. Thoory	BLDG				
cv. Zahidi	USDCS	2.1 : .78	.2 : .2		
dactylifera x *reclinata*	USDCS	1.6 : .9	.25 : .13	--	--
hamefarra formosana	APS	1.0 : .7	.24 : .15	--	Tropical Taiwan.
humilis Royle				Solitary trunk; short stature; dioecious; pinnate leaf.	Tropical China.
pusilla Gaertn.	LASCA	1.6 : .8	.18 : .08	Multiple trunks; short stature; dioecious; pinnate leaf.	Tropical Southern India and Sri Lanka
reclinata Jacq.	USDCS	1.2 : .6	.15 : .1	Multiple trunks; medium stature; dioecious; pinnate leaf.	Tropical Africa.
Phoenix					
roebellenii O'Brien	APS	1.3 : .5	.12 : .05	Solitary and multiple trunks; dwarf stature; dioecious; pinnate leaf.	Tropical China.
rupicola Anders.	LASCA	1.75 : .8	.2 : .16	Solitary trunk; medium stature; dioecious; pinnate leaf.	Tropical India.
sylvestris (L.) Roxb.	APS	2.0 : 1.2	.25 : .13	do.	Do.
Pigafetta					
filaris Harkes	UH	.7 : .5	.1 : .05	Solitary trunk; tall stature; monoecious; pinnate leaf.	--

Table 3--Continued
List of palm members used in tissue-culture study

Name	Seed or plant source[1]	Size (cm)[2] Seed	Size (cm)[2] Embryo	Habit	Distribution
Pinanga copelandii Becc.	APS	1.2	.14 : .10	Multiple slender trunks; medium stature; monoecious; pinnate leaf.	Tropical Southeast Asia and Philippines.
Prestoea sp.	LASCA	.8	.22 : .13	Multiple slender trunks; dwarf stature; monoecious; pinnate leaf.	Tropical Central America and West Indies.
Ptychosperma microcarpum Wendl.	APS	1.14 : .96	.5 : .37	--	--
Rhapalostylis sapida H. Wendl. and Druce	APS	1.2 : .8	.15 : .1	Solitary trunk; medium stature; monoecious; pinnate leaf.	Subtropical New Zealand.
Sabal domingensis Becc.	LASCA	1.3 : .5	.21 : .11	Multiple trunks; subterranean-tall stature; monoecious; palmate leaf.	Tropical Cuba.
etonia Blatter	APS	1.01 : .4	.1 : .05	Multiple trunks; subterranean stature; monoecious; palmate leaf.	North America.
minor (Jacq.) Pers.	APS	.4	.1 : .05	Multiple trunks; subterranean-tall stature; monoecious; palmate leaf.	Tropical North America and West Indies.
palmetto Becc.	UF	.65	.02 : .05	Solitary trunk; tall stature hermaphrodite; palmate leaf.	North America.

Thrinax radiata Lodd. ex Desf.	APS	.7	.13 : .07	Solitary trunk; tall stature; hermaphrodite; palmate leaf.	Tropical North America and West Indies.
Trachycarpus fortunei (Hock.) H. Wendl.	APS	1.2	.18 : .1	Solitary trunk; variable stature; monoecious; palmate leaf.	Tropical China and Japan.
Veitchia merrillii (Becc.) Moor.	APS	2.5 : .77	.21 : .65	Solitary trunk; medium stature; monoecious; pinnate leaf.	Philippine Islands.
Washingtonia filifera (Linden ex Andre) H. Wendl.	APS	1.0 : .6	.34 : .13	Solitary slender trunks; tall stature; hermaphrodite; palmate leaf.	Subtropical North America (California).
robusta Wendl.	APS	1.1 : .6	.29 : .13	do.	Do.

[1]Seeds and plant material were obtained from the following sources: American Palm Society (APS), Los Angeles State and County Arboretum (LASCA), U.S. Date and Citrus Station (USDCS), Botanical Gardens of Singapore (BGS), University of Hawaii (UH), University of Florida, Ft. Lauderdale (UF), United Fruit Company (UFC), Ben Laflin Date Gardens (BLDG), and local nurseries (LN) or market (LM).

[2]Specimen sizes: diameter or length: width measurements for spherical and elongated specimens, respectively.

Table 4.
Morphogenetic response obtained from palm embryos planted on 2 types of nutrient medium

Name	Morphogenetic responses (%)					
	Nutrient medium, only[1]			Nutrient medium + 2,4-D[2]		
	Initial culture	1st culture	2d culture	Initial culture	1st culture	2d culture
Aiphanes caryotifolia	CE,23	CE,23	CE,23	0	0	0
Archontophoenix alexandrae	C,100	C,100;R,10;G,10	C,100;R,10;G,10	CE,30	G,40;R,20	G,40;R,20
Archontophoenix cuninghamiana	C,30	C,30	C,30	G,20;CE,10	G,20;CE,10	G,20;CE,10
Arecastrum romanazoffianum	C,60	C,60;CE,40	C,60;CE,40	G,40	G,40;R,40;AR,40	G,45;R,45;AR,45
Arenga mindorensis	CE,35	CE,40	CE,40	CE,60	CE,65;R,65	CE,65;R,65
Brahea armata	C,75	C,75	C,75;AE,5	G,70	G,70;R,70;AR,70	G,70;R,70;AR,70
Brahea dulcis	C,25;CE,53	C,25;CE,53	C,25;C,53;AE,7	CE,75	CE,80;R,80	CE,80;R,80
Butia capitata	C,43	C,45;G,15	C,35;G,15	G,14	G,50;R,50;AR,50	G,50;R,50;AR,50
Caryota mitis L.	C,20	C,60	C,60	CE,90	CE,80;G,10	CE,80;G,10
Chamaedorea glaucifolia	C,10;CE,20	C,10;CE,20	C,10;CE,20	CE,10	G,50;R,50	G,50;R,50
Chamaedorea humilis	CE,65	CE,73	CE,73	CE,70	CE,70	CE,70
Chamaedorea radicalis	CE,45	CE,45	CE,47	CE,50	CE,50	CE,50;R,50;AR,50
Chamaedorea sp.	C,10;CE,50	C,10;CE,50	C,10;CE,50;R,30	0	0	0

Chelyocarpus thindera	CE,75	CE,77	CE,77	CE,80	CE,80;R,80;AR,25	CE,80;R,80;AR,65
Chrysalidocarpus lutescens	C,66	C,66	C,66	0	0	0
Cocos nucifera	0	C,70	C,66	0	0	0
pulposa	C,30	C,40	C,40	CE,100	G,100;R,70	G,100;R,70
Corypha elata	C,47	C,50	C,50	G,30	G,40;R,40	G,40;R,40
Dictyocaryum platysepalum	C,10	C,10	C,10	0	0	0
Elaeis quineensis	C,40	C,100;CE,40	C,100;G,100;R,100	CE,50	CE,50	CE,50
Erythea edulis	C,55	C,55;G,16	C,55;G,16	G,50	G,55;R,55;AR,55	G,65;R,65 AR,65
Heterospathe elata	G,70	G,70;R,70;AR,70	G,70;R,70;AR,70	C,69	C,72	C,72
Hydriastele sp.	CE,60	CE,80	CE,80	C,40	C,40;G,30;R30	C,40;G,30; R,30
Howia forsteriana	0	0	0	C,53	C,53	C,53
Licuala spinosa	0	C,30	C,30;CE,20	0	C,30	C,30;CE,20
Livistona decipiens	G,70	G,70;R,70;AR,70	G,70;R,70;AR,70	C,65	C,66;G,23	C,66;G,23; AE,7
merrillii	0	0	0	C,34	C,34	C,34
robinsoniana	CE,60	CE,20;G,40	G,40;CE,20	C,100	C,100	C,100
saribus	G,50	G,50;R,50;AR,50	G,50;R,50;AR,50	0	0	0

Table 4--Continued
Morphogenetic response obtained from palm embryos planted on 2 types of nutrient medium

Name	Morphogenetic responses (%)					
	Nutrient medium, only[1]			Nutrient medium + 2,4-D[2]		
	Initial culture	1st culture	2d culture	Initial culture	1st culture	2d culture
Normanbya						
normanbyi	0	0	0	C,10	C,20	C,20
Onocosperea						
horridum	CE,30	CE,60	CE,60	CE,60	CE,83	CE,83
Opsiandra						
maya	G,65	G,65;R,65;AR,65	G,65;R,65;AR,65	C,69	C,69	C,69
Phoenix						
canariensis	CE,75	CE,85;R,85	CE,85;R,85	C,53	C,53	C,53
dactylifera						
cv. Barhee	CE,80	CE,20;G,80	G,80;CE,20	C,20	C,20	C,20
cv. Deglet Noor	G,50	G,50;R,50;AR,50	G,50;R,50;AR,50	C,61	C,61	C,61;AE,14
cv. Medjool	CE,50	G,100;R,100	G,100;R,100	C,100	C,100	C,100
cv. Sayer	CE,80	G,100;R,100	G,100;R,100	C,43	C,45	C,45;AE,11
cv. Zahidi	CE,100	G,100;R,100	G,100;R,100	C,100	C,100	C,100
hamefarra						
formosana	CE,80	CE,85;R,85	CE,85;R,85;AR,13	CE,73	CE,75	CE,75
pusilla	G,53	G,60;R,60;AR,60	G,60;R,60,AR,60	C,64	C,65	C,65;AE,10
reclinata	0	0	0	C,31	C,31	C,31
rupicola	CE,100	CE,50;G,50;R,50	G,50;CE,50;R,50	C,100	C,100	C,100
sylvestris	CE,100	CE,100;R,60	CE,100;R,60	C,25	C,25	C,25;AE,9
Pigafetta						
filaris	CE,70	CE,70;R,20	CE,70;R,20	C,20;CE,50	C,20;CE,60 G,20	C,20;CE,60; G,20
Pinanga						
copelandii	CE,60	CE,67;R,67	CE,87;R,87;AR,11	0	0	0

Prestoea sp.	CE,40	CE,40;R,40	CE,40;R,40	C,35	C,35	C,35;AE,14
Ptychosperna microcarpum	G,20	G,20	G,20	C,10;CE,50	C,10;CE,30;G,20; R,10	C,10;CE,30; G,20;R,10
Rhapalostylis sapida	CE,70	CE,73;R,73	CE,73;R,73	C,42	C,43	C,43
Sabal domingensis	CE,71	CE,79;R,79	CE,79;R,79	CE,65	CE,65	CE,65
etonia	CE,20	CE,20	CE,20;R,10	CE,100	CE,30;C,40;G,20; R,10	CE,30;C,40 G,20;R,20
minor	0	0	0	C,20	C,40;G,40;R,10	C,40;G,40; R,40
palmetto	CE,10	CE,20	CE,20	0	0	0
Thrinax radiate	G,45	G,65;R,65;AR,65	G,65;R,65;AR,65	C,36	C,38;G,52	C,38;G,52
Trachycarpus fortunei	G,30	G,40;R,40	G,40;R,40	CE,40	CE,42	CE,42
Veitchia merrillii	CE,30	G,40;R,40	G,50;R,40	C,10	C,60;CE,10	C,60;CE,10
Washingtonia filifera	G,79	G,85;R,85;AR,85	G,85;R,85;AR,85	C,25	C,25	C,25
robusta	G,40	G,50;R,50	G,50;R,50	0	0	0

[1]Responses observed: G, germination to form a seedling; AR, adventitious root formation from seedling; CE, cotyledon elongation; and R, emergence or elongation of primary root only from elongated cotyledon or seedling.

[2]Responses observed: C, callus formation; CE, cotyledon elongation; AE adventitious embryo production via callus; G, germination to form a seedling and R, root formation.

Table 5.
Morphogenetic responses obtained from shoot-tip cultures of various arborescent monocotyledonous species

Plant	Media composition[1]			
	+2,4-D		+NAA	
	8 wks	32 wks	8 wks	32 wks
Archontophoenix cunninghamina	NC[2]	NC	--	--
Brahea armata	NC	FC,NC	--	--
Chamedorea excelsa	0	0	SE,LD	--
Corphya elata	NC	FC,NC	--	--
Erythea edulis	NC	FC,NC,AE	SE,LD	SE,LD, AR,FLP
Metroxylon sp.	SE,LD	SE,LD,AB,NC, FC,AE		
Phoenix canariensis	NC	NC,FC,AE	SE,LD	SE,LD, AR,FLP
canariensis x dactylifera	NC	NC,FC	0	0
dactylifera				
cv. 'Khalasa'	SE,LD,NC	NC,FC,AE	SE,LD	SE,LD, AR,FLP
cv. 'Thoory'	SE,LD,NC	NC,FC,AE	SE,LD	SE,LD, AR,AB, FLP
cv. 'Zahidi'	SE,LD,NC	NC,FC,AE	SE,LD	SE,LD, AR,FLP
humilis	SE,LD,NC	FC,NC	--	--
reclinata	0	0	SE,LD	--
roebilinii	SE,LD,NC	SE,LD,NC,FC AE	0	0
sylvestris	SE,LD,NC	FC,NC,AE	--	--
Sabal minor	SE,LD,NC	SE,LD,FC,NC	--	--

Table 5--Continued
Morphogenetic responses obtained from shoot-tip cultures
of various arborescent monocotyledonous species

Plant	Media composition[1]			
	+2,4-D		+NAA	
	8 wks	32 wks	8 wks	32 wks
Trachycarpus fortunei	SE,LD	SE,LD	--	--
Washingtonia filifera	SE,LD	SE,LD,FC,	SE,LD	SE,LD, AR,FLP
robusta	SE,LD	SE,LD,FC	SE,LD	SE,LD, AR,FLP

[1]Explants cultured on a basal nutrient medium containing Murashige and Skoog inorganic salts, 3 percent sucrose, 0.4 mg/L thiamine•HCl, 100 mg/L i-inositol, 0.8 percent Phytagar and either 100 mg/L 2,4-dichlorophenoxyacetic acid, 3 mg/L 2iP and 0.3 percent activated charcoal (+2,4-D) or 0.1 mg/L α-naphthaleneacetic acid (+NAA). 3 to 15 explants were employed per species.

[2]Growth response obtained are designated as follows: 0, no growth occurred; --, test not conducted; SE, shoot elongation; LD, leaf differentiation; AR, adventitious root formation; FLP, formation of a free-living palm that was transferred to soil; AB, production of axillary branches; NC, formation of nodular callus; FC, formation of friable callus; AE, formation of asexual embryos from callus.

Figure 1.
Techniques used for field vegetative propagation of the date palm: A, Adult palm with basal suckers; B, severing vascular connection with chisel and sledge hammer; C, prying shoot loose from parent tree; D, detached shoot that may be planted directly in the field; E, an offshoot wrapped with a burlap bag and planted in the field.

Figure 2.
Excising date palm shoot tip through chain-saw cutting: A, leaves of palm are trimmed off and a shallow cut is made around the shoot-tip region; B, shoot-tip region with outward debris removed; C, using a sharp knife, petiole and leaf segments are then peeled and cut away; D, exposed shoot tip; E, shoot tip is excised through a transverse cut made well below the apex. Excised shoot tip is then placed in antioxidation solution.

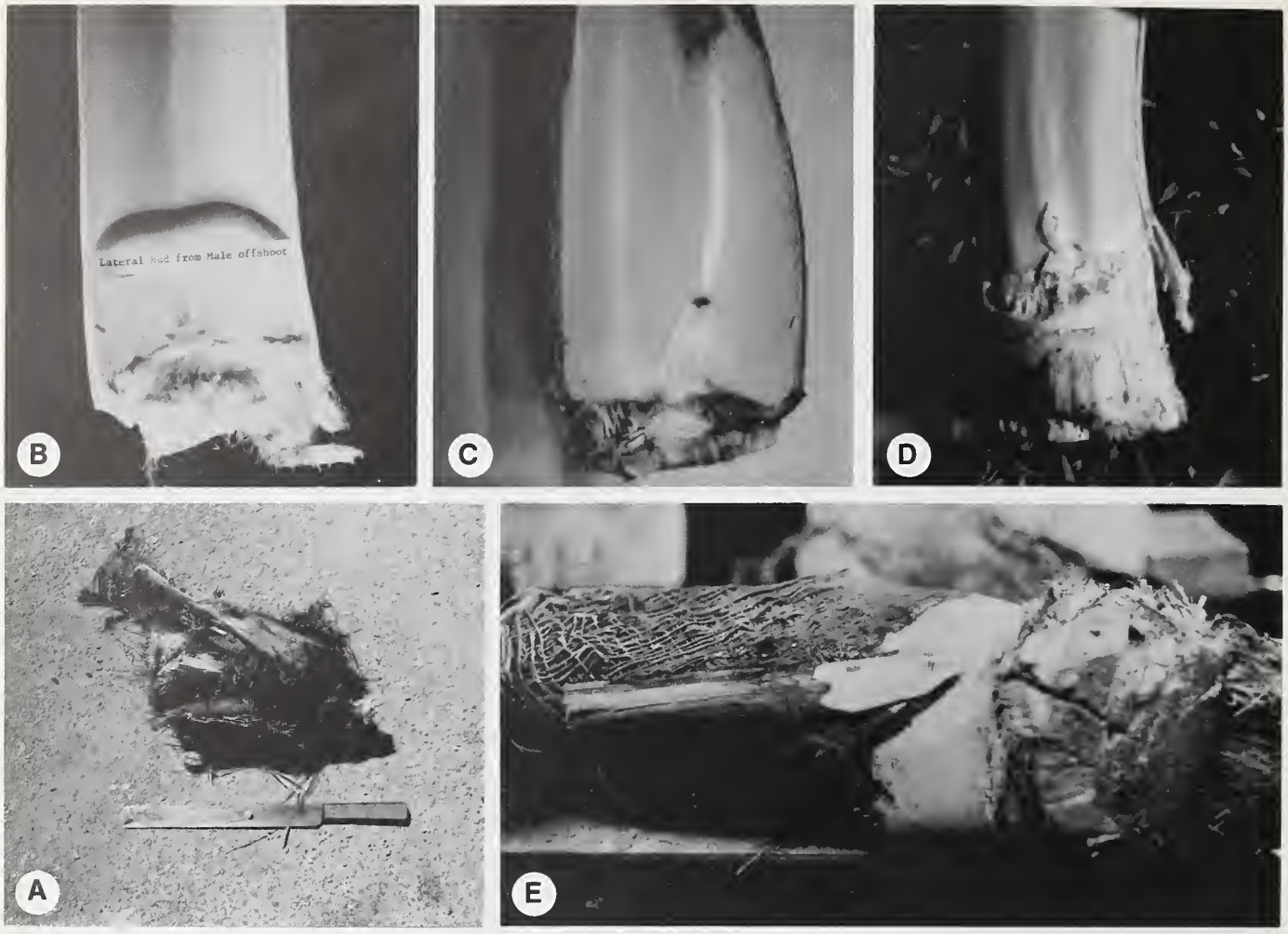


Figure 3.
Examples of lateral buds found within cut date palm offshoots: A, an offshoot cut with a chain saw; Note white dot on knife shows amount of shoot-tip tissue that will be excised to be planted in vitro from this shoot; B, leaf with undifferentiated lateral bud, such buds rarely survive in vitro; C, and D, leaves with differentiated lateral buds that will grow well in vitro; E, a well-differentiated lateral bud exhibiting senescence.

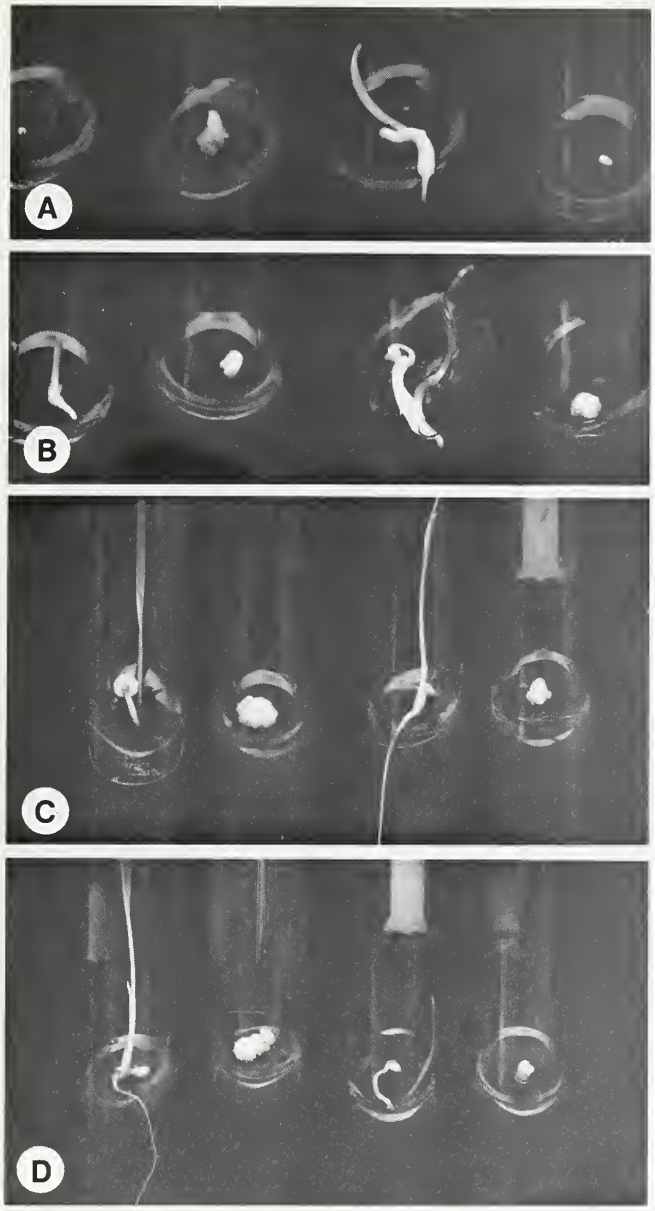
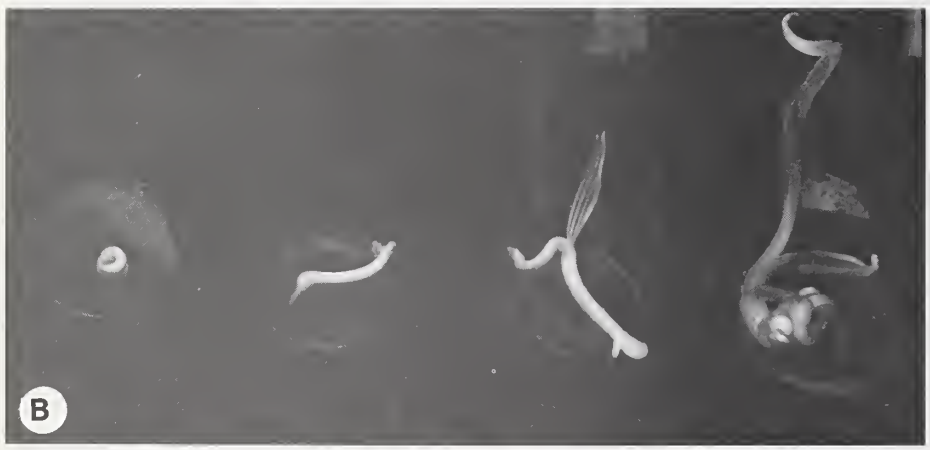


Figure 4.
Comparison of excised palm embryo growth responses after 8 wks on nutrient medium containing 0.3 percent charcoal: A, Left to right: Cultures of *Archontophoenix cunninghamiana* on 0 and 100 mg/L 2,4-D and 3 mg/L 2iP and *Trachycarpus fortunei* on 0 and 100 mg/L 2,4-D and 3 mg/L 2iP. B, cultures of *Sabal minor* on 0 and 100 mg/L 2,4-D and 3 mg/L 2iP and *Prestoea* sp. on 0 and 100 mg/L 2,4-D and 3 mg/L 2iP; C, cultures of *Erythea edulis* on 0 and 100 mg/L 2,4-D and 3 mg/L 2iP and *Trachycarpus fortunei* on 0 and 100 mg/L 2,4-D and 3 mg/L 2iP; D, cultures of *Phoenix pusilla* on 0 and 100 mg/L 2,4-D and 3 mg/L 2iP and *Brahea armata* on 0 and 100 mg/L 2,4-D and 3 mg/L 2iP.



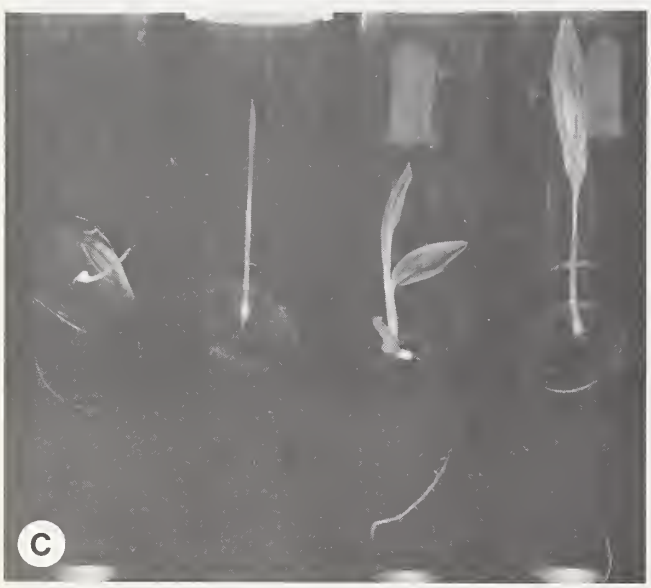


Figure 5.
Variation in palm-seedling development from excised embryos after 16 weeks in culture on a nutrient medium containing 0.1 mg/L NAA: A, Heterospathe elata; B, Phoenix dactylifera; and C, Corypha elata.

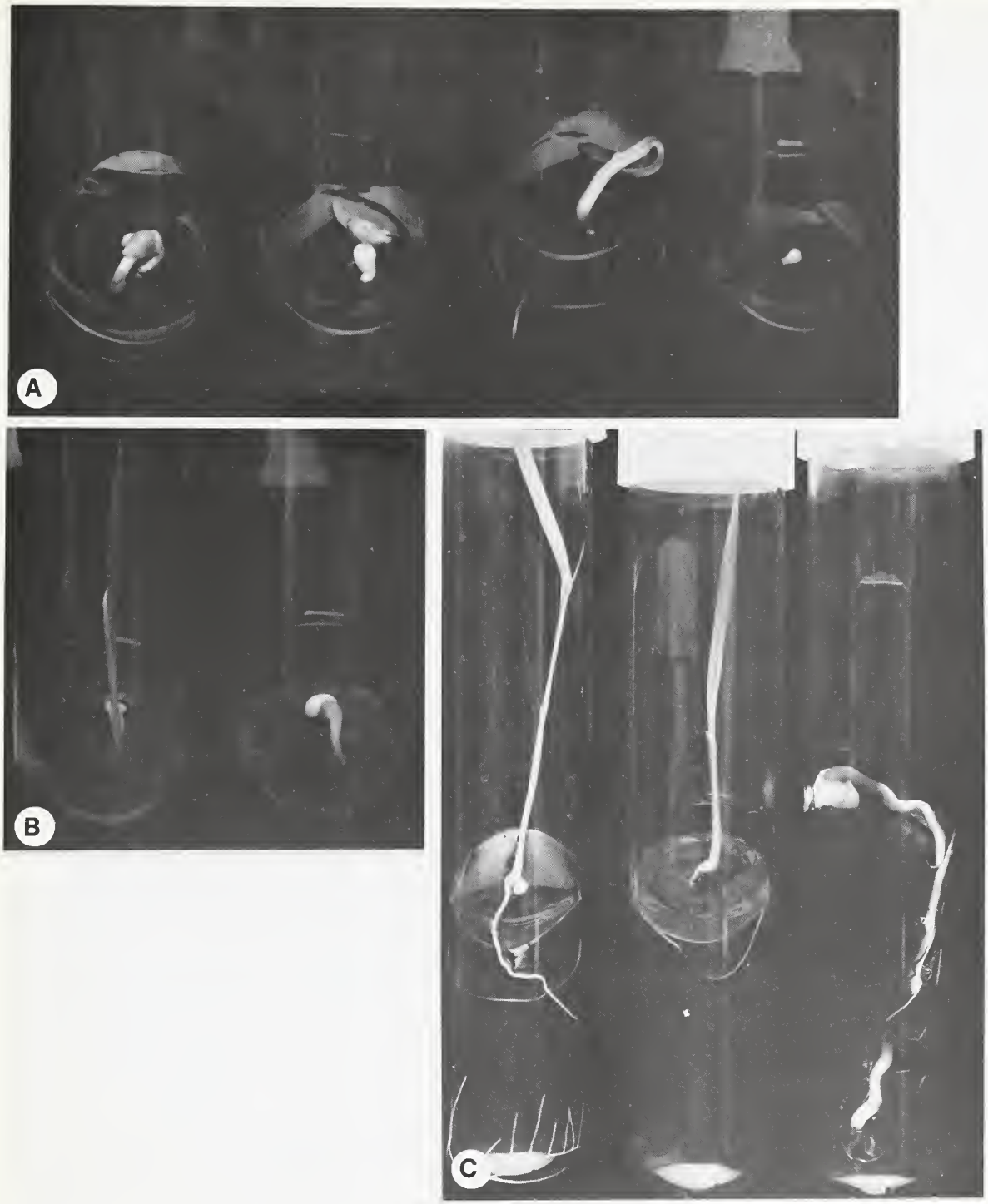


Figure 6.
Development of excised embryos from various palm species cultured for 8 wks. on a nutrient medium containing 0.3 percent charcoal, left to right: A, *Opsiandra maya*, *Rhapalostylis sapida*, *Phoenix dactylifera* cv. 'Sayer', and *P. reclinata*; B, *Thrinax radiata* and *Livistona saribus*; C, *Washingtonia filifera*, *Heterospathe elata*, and *Livistona merrillii*.

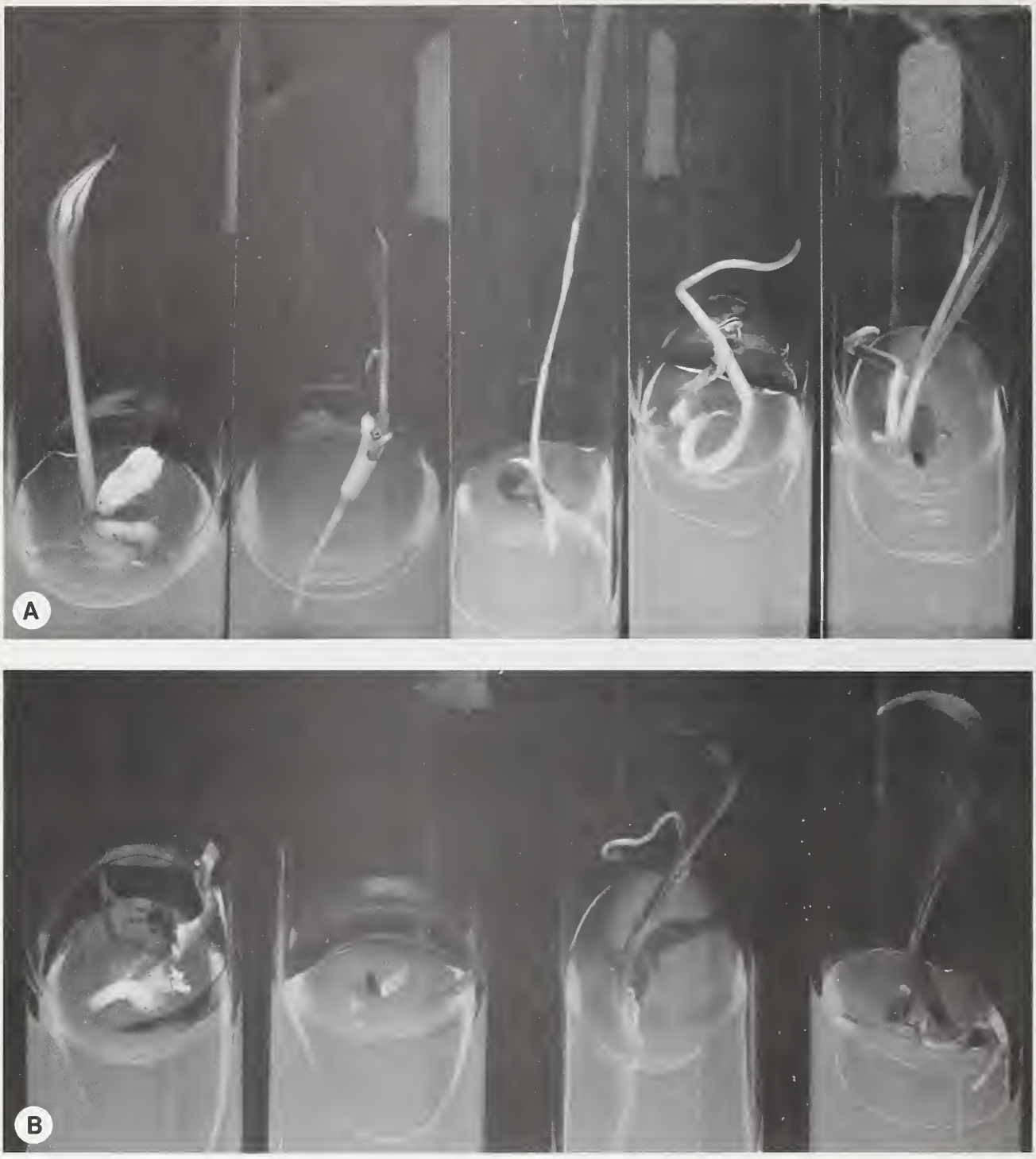


Figure 7.
Growth of 16-wk-old seedlings from germinated zygotic embryos cultured for 8 wks on basal nutrient medium containing 0.1 mg/L NAA, left to right: A, Erythea edulis and Butia capitata, Brahea armata, Phoenix dactylifera cv. 'Deglet noor' and P. pusilla; B, Phoenix dactylifera cv 'Sayer", Sabal minor, Prestoea sp., and Phoenix pusilla.

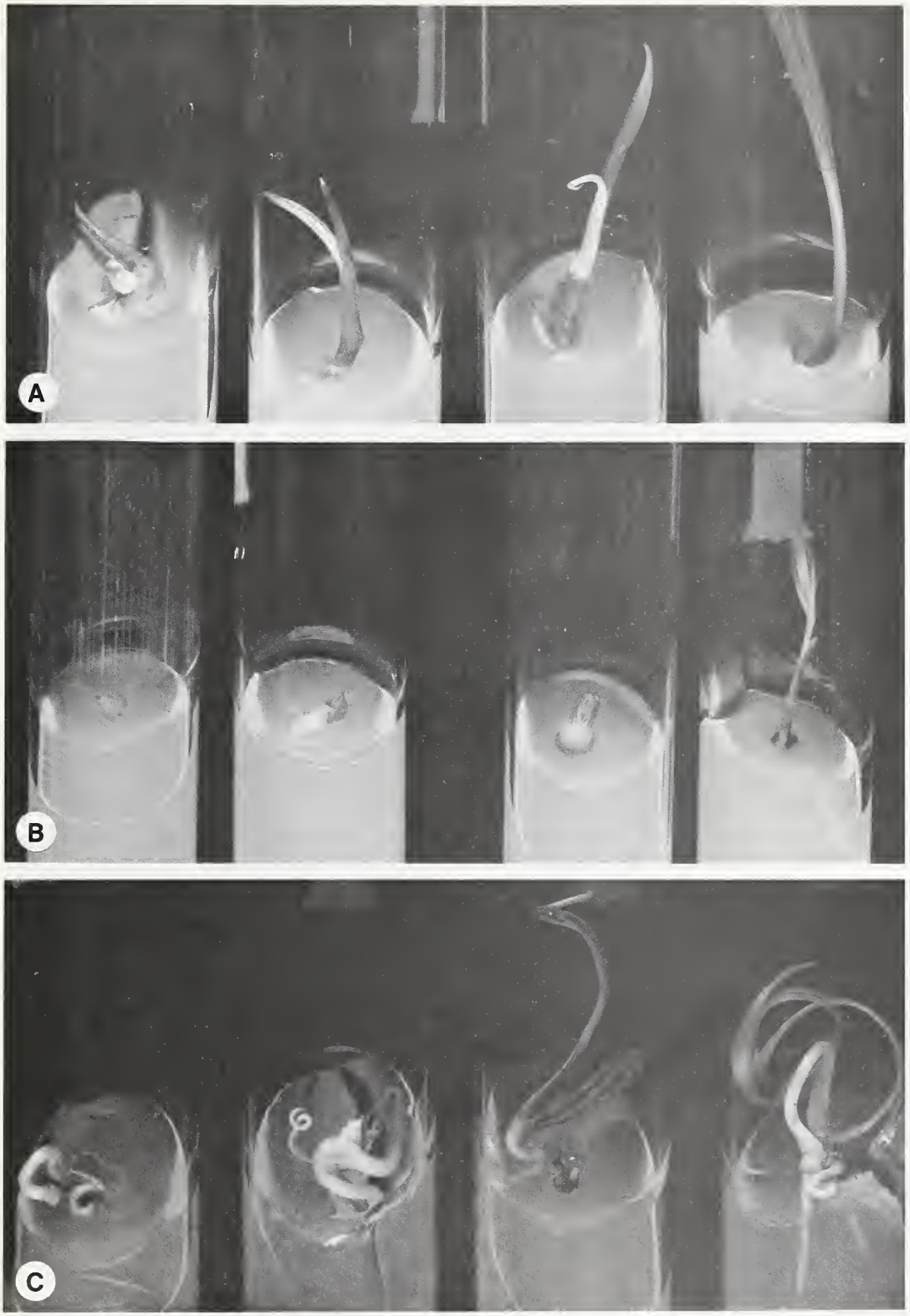
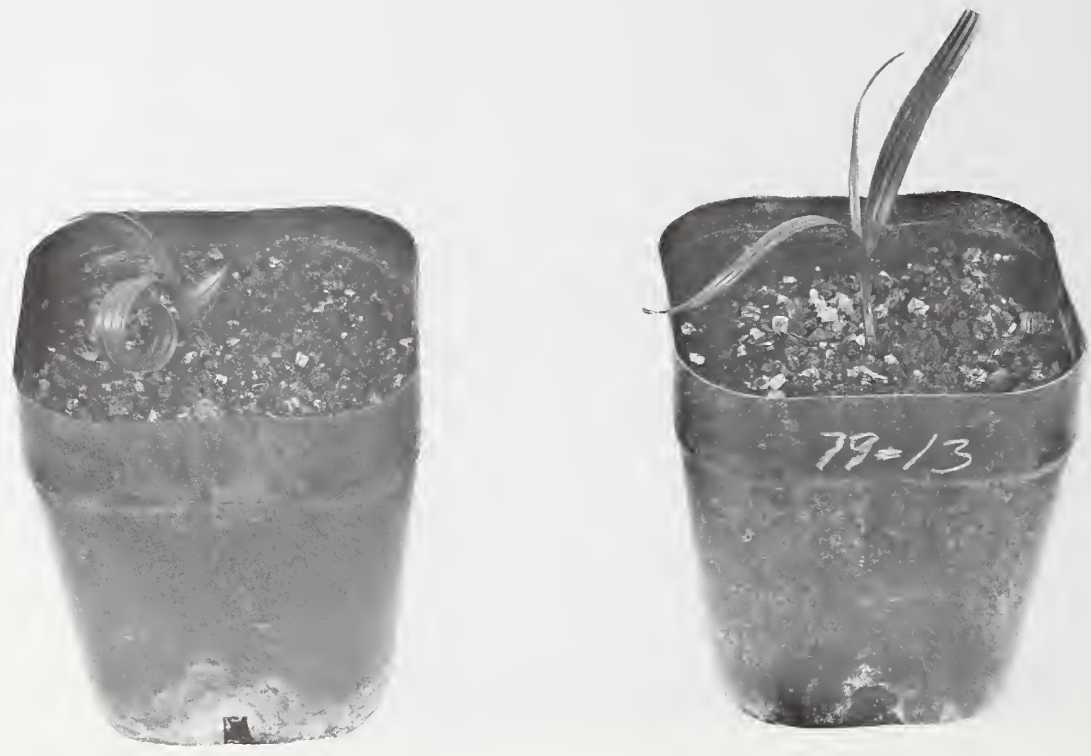


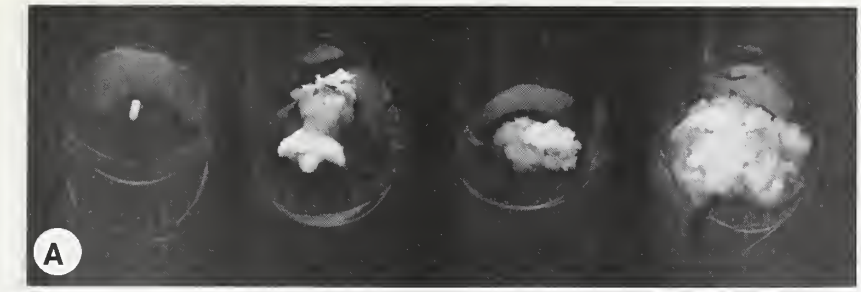
Figure 8.
Variation in excised embryo development after 8 wks cultured on nutrient medium containing 0.3 percent charcoal; A, Opsiandra maya, B, Phoenix dactylifera cv. 'Deglet noor', C, Heterospathe elata. Note wide variety of growth responses.

A



B

Figure 9.
Free-living palms derived from excised embryo cultures, left to right: A, Thrinax radiata, Washingtonia filifera, and W. robusta. Palms are about 3 months old; B, 10-month-old date palm seedlings.



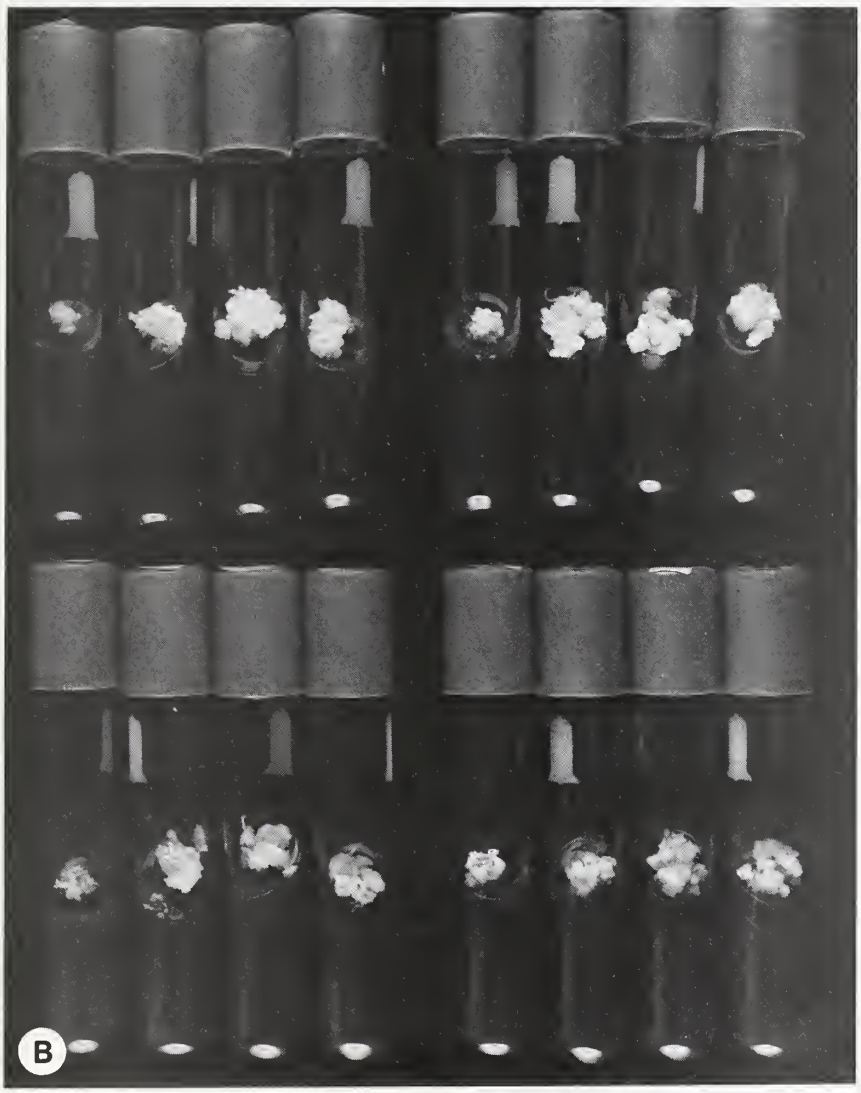


Figure 10.
Examples of callus formation in palms derived from excised embryos on nutrient medium containing 0.3 percent charcoal, 100 mg/L 2,4-D, and 3 mg/L 2iP after 24 wks. in culture, left to right: A, Aiphanes caryotifolia, Phoenix dactylifera cv. 'Deglet noor', Brahea armata, and P. pusilla; B, Erythea edulis (top left); Brahea armata (top right); Livistona decipiens (bottom left); and Phoenix dactylifera cv. 'Sayer' (bottom right).

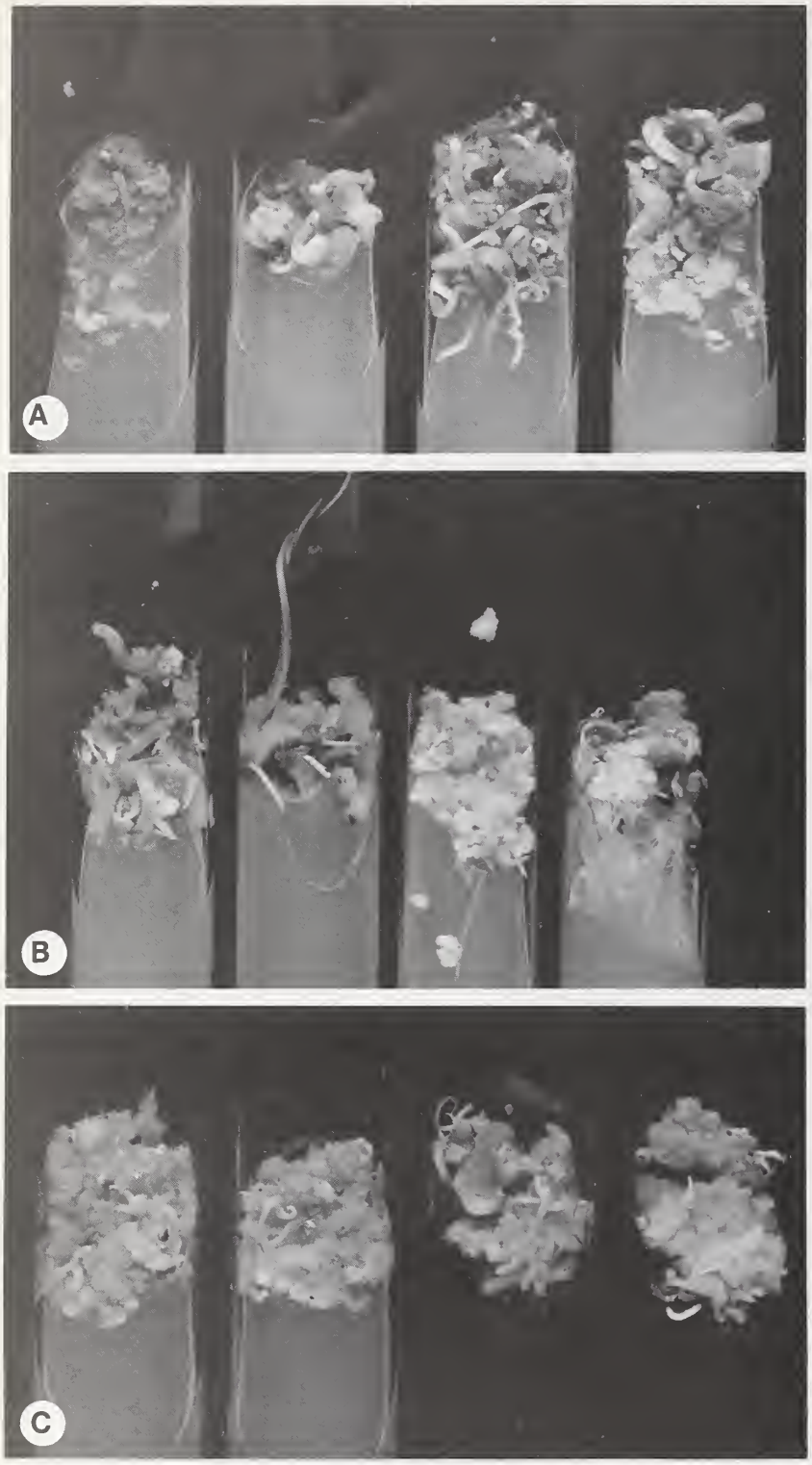


Figure 11.
Examples of embryogenic callus derived from excised embryos of various palm species. Callus was initially cultured on nutrient medium containing 100 mg/L 2,4-D, 3 mg/L 2iP, and 0.3 percent charcoal then transferred to medium devoid of hormones: A, Livistona decipiens; B, Brahea armata; and C, Erythea edulis.

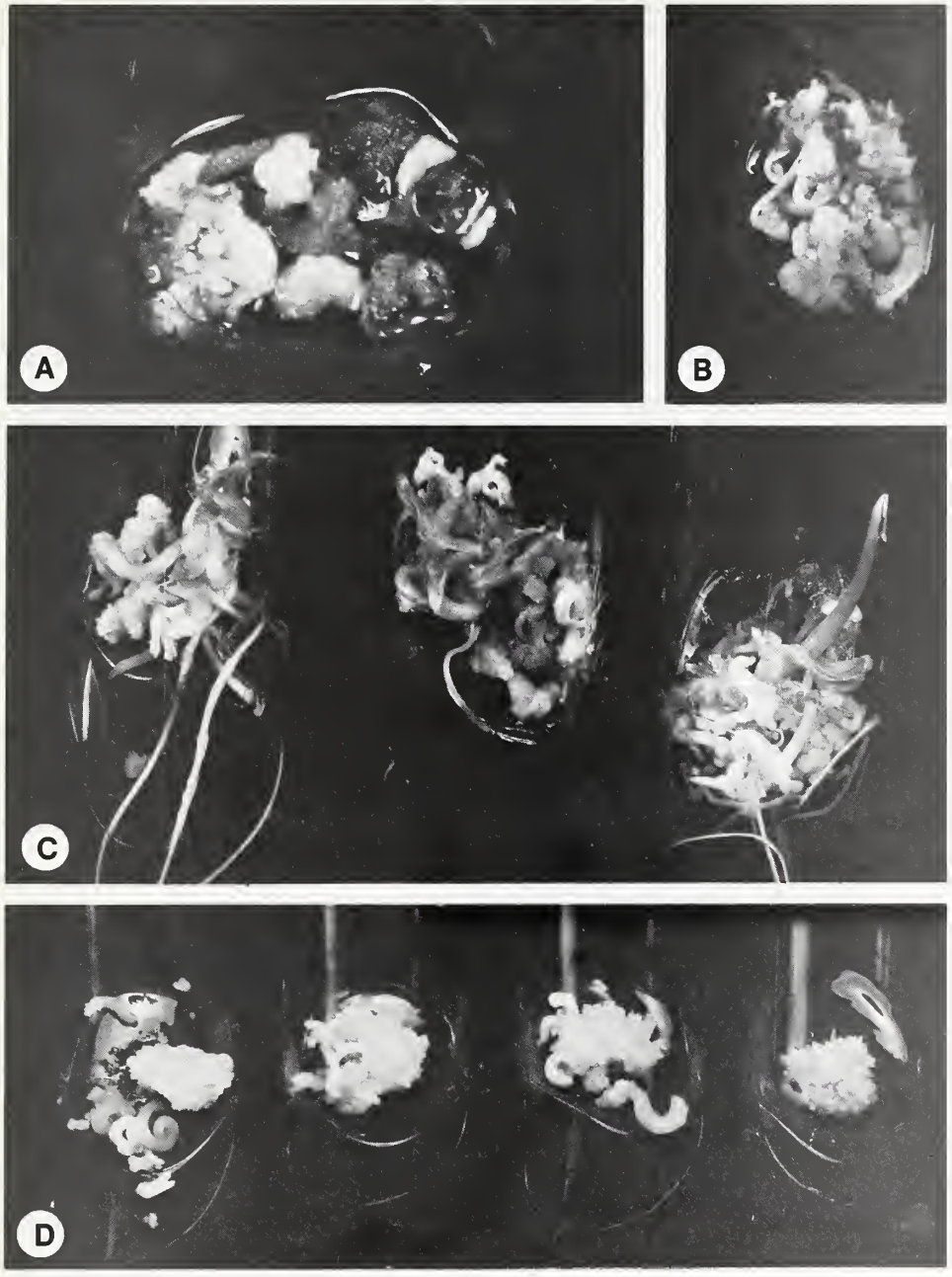


Figure 12.
Example of callus types, embryo, and plantlet formation from date palm shoot-tip cultures: A, initial nodular callus, which will give rise to asexual embryos derived from a lateral bud culture, after about 4-months old; B, further initiation of nodular callus and asexual embryos after 6 months of culture, C, subcultured, fast-growing, friable/ nodular callus initiating asexual embryos following 8 months of culture; D, growth responses obtained from another isolated callus clone derived from the same lateral bud culture.

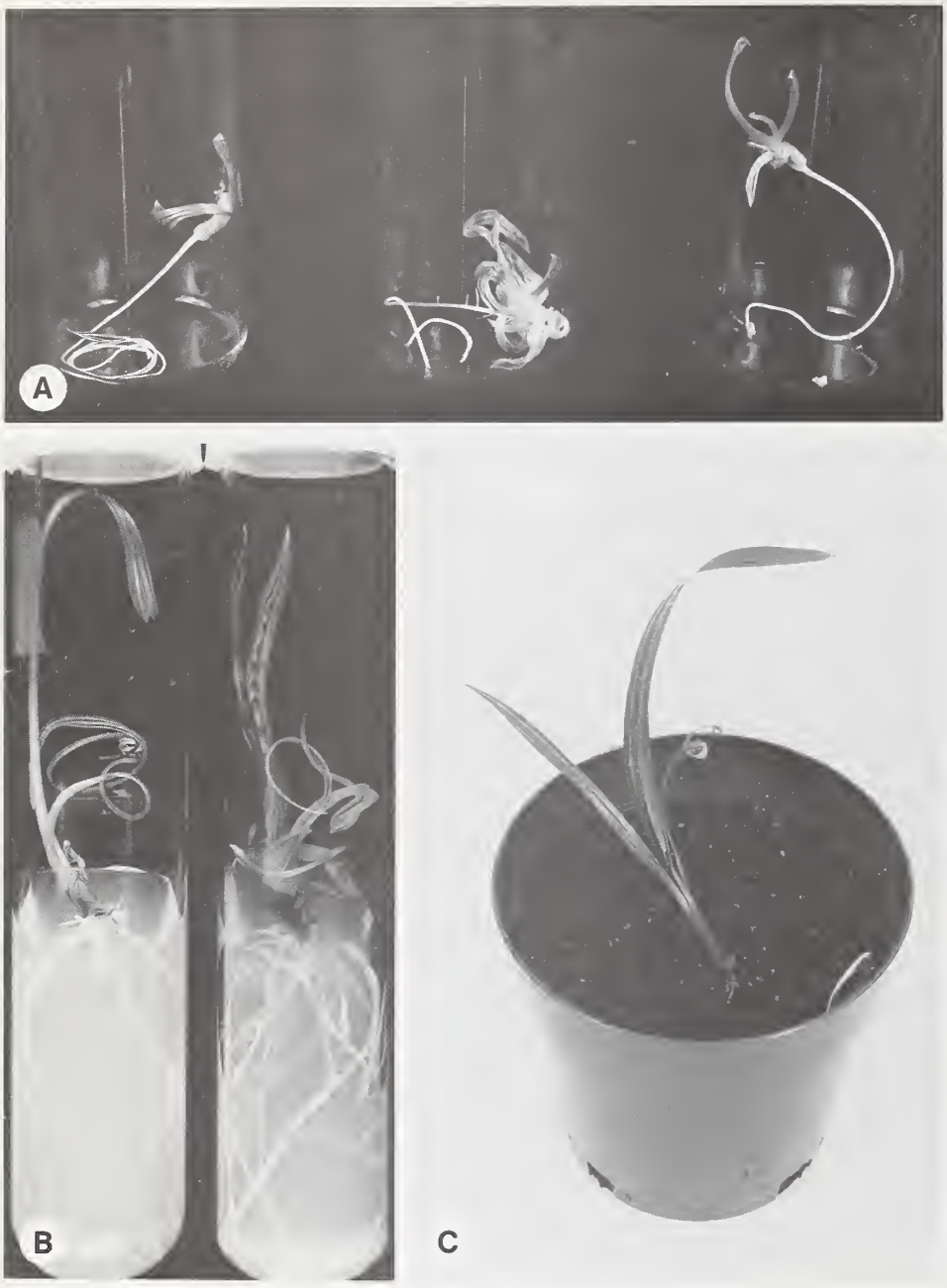


Figure 13.
Asexual plantlets produced from date palm callus: A, plantlets isolated from callus--note occurrence of long primary roots; because of the size of the plantlet with intact root system, transferring to fresh medium is tedious; B, asexual plantlets with well-developed adventitious root systems. Primary root was cut off and shoot planted in 0.1 mg/L NAA for 8 weeks. These plantlets may be successfully transferred to soil; C, free-living palm derived from an asexual embryo. Plantlet has been in soil for about 4 wks.

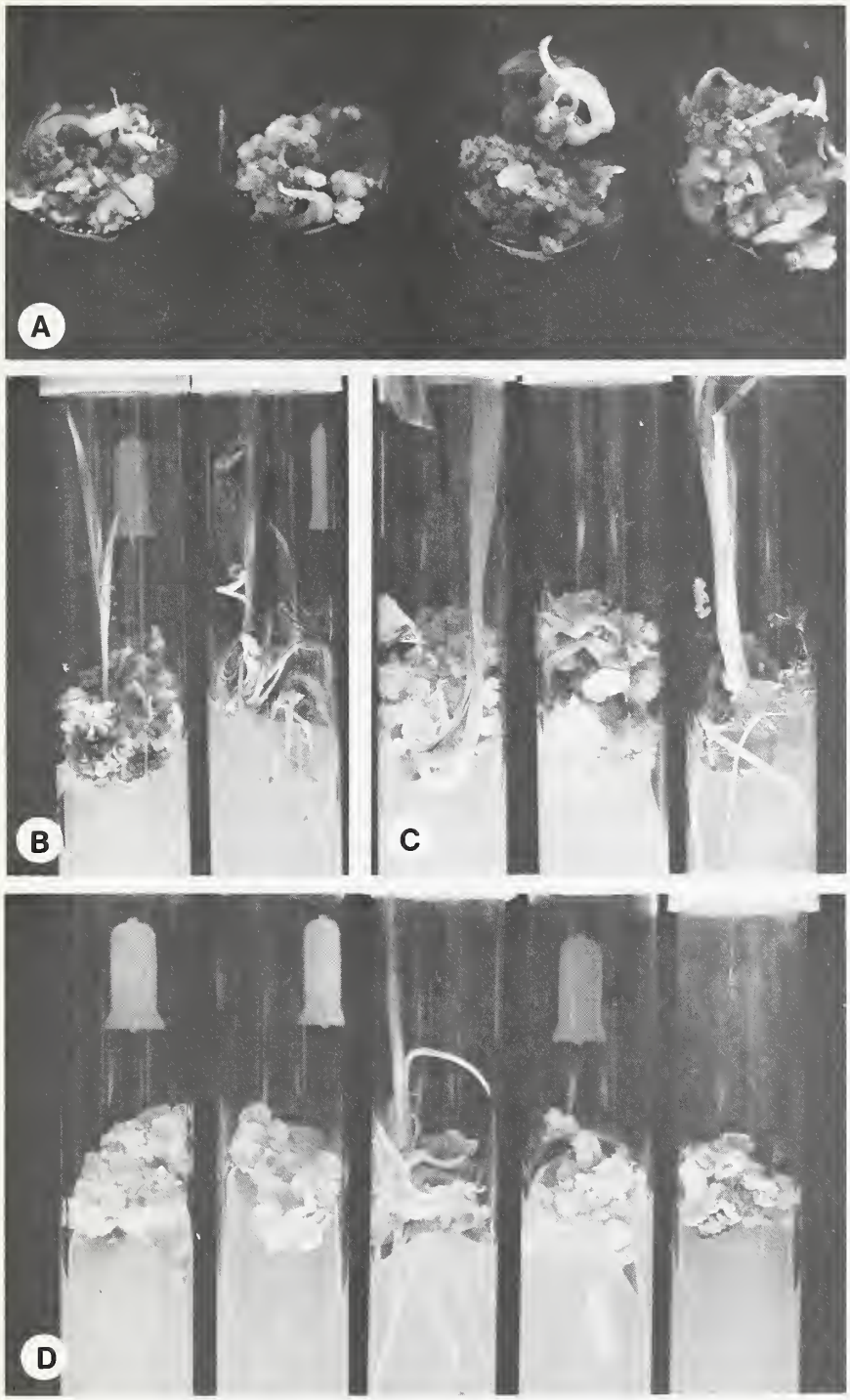


Figure 14
Embryogenic callus derived from shoot tips of various palm species: Callus was initiated from tips cultured on nutrient medium containing 100 mg/L 2,4-D, 3 mg/L 2iP, and 0.3 percent charcoal, then transferred to medium devoid of hormones: A, *Phoenix dactylifera*, left to right: cultivars 'Zahidi', 'Thoory', 'Thoory', and 'Halawy'; B, *Phoenix pusilla*, C, *Erythea edulis* and D, *Phoenix humilis*.

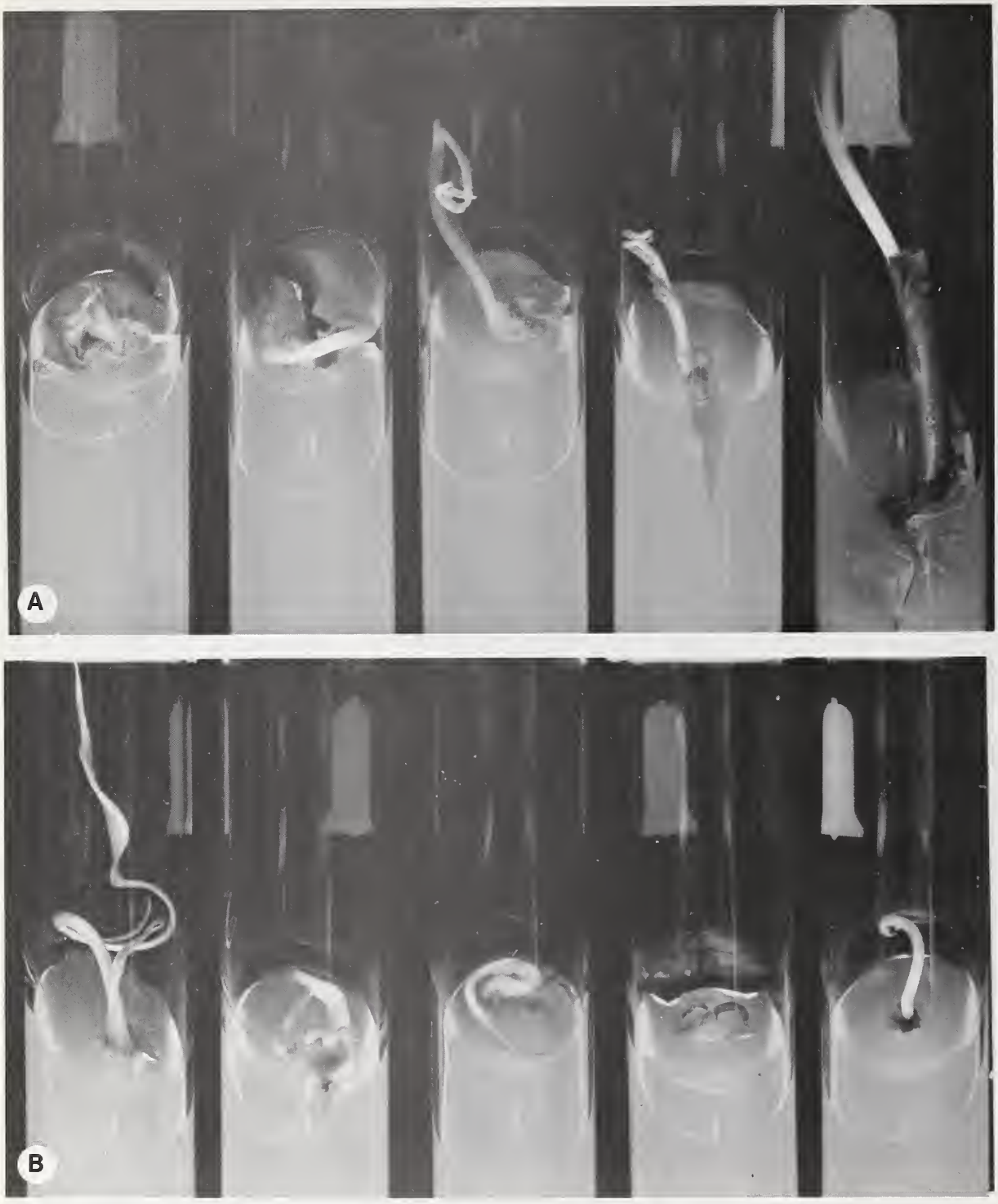


Figure 15.
Examples of growth obtained from shoot tips of palm species: The tips, about 8-wks old, were cultured initially on nutrient medium containing 0.1 mg/L NAA. A (left to right): Phoenix sylvestris, middle three cultures are P. reclinata and P. dactylifera; B, variation observed in Sabal minor tips.

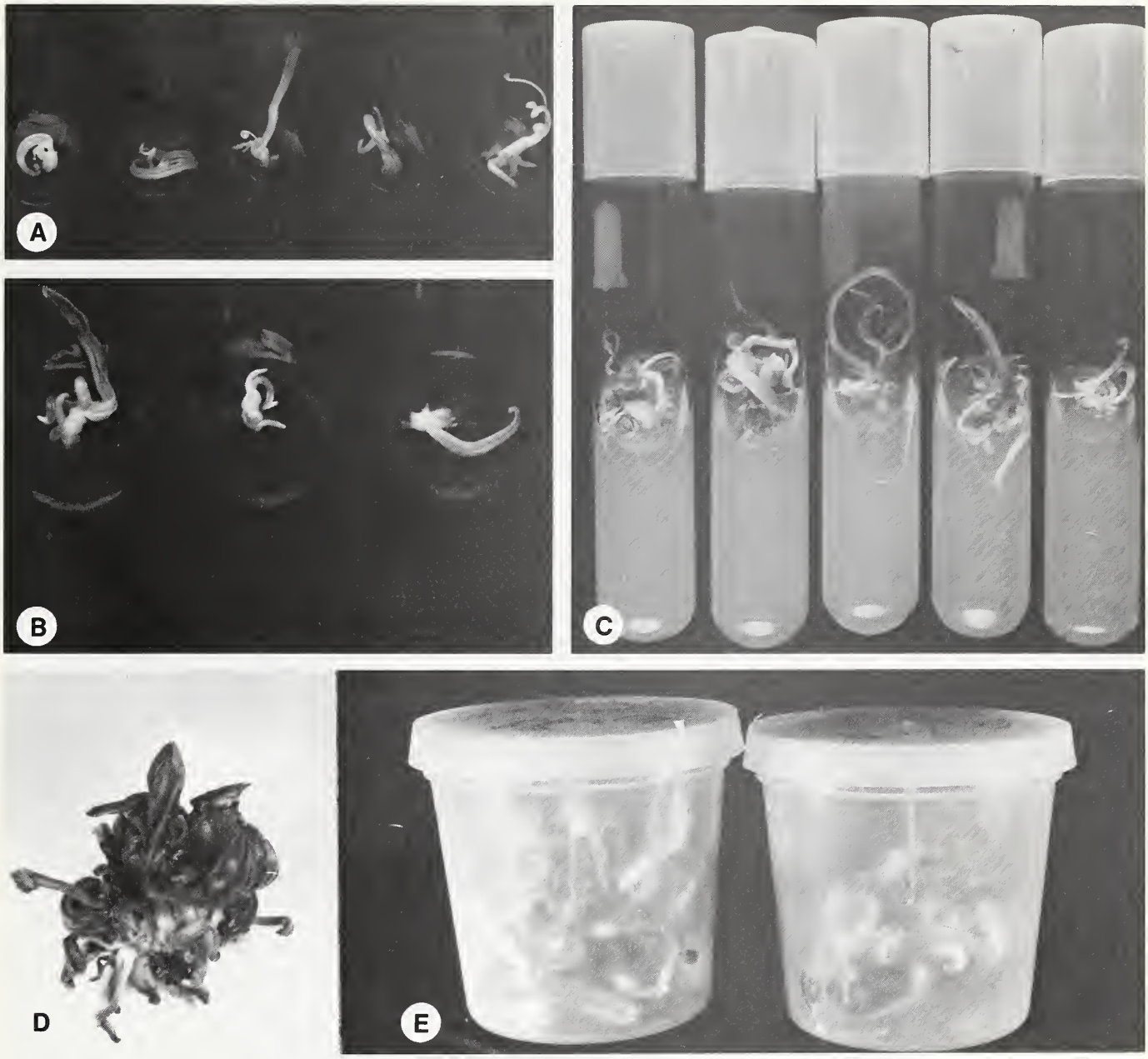


Figure 16.
Shoot-tip culture in date palm: A, 8-wk-old growing shoots derived from tips consisting of the apical meristem and a few leaf primordia; B, early formation of additional lateral shoots from 8-wk-old tips. Additional shoots are projections emerging from base of shoot; C, enlargement of additional shoots, about 16-wk-old, on nutrient medium containing 0.1 mg/L NAA; D, isolated 24-wk-old shoot tip cultured consisting of numerous twisted axillary shoots; E, shoot cultures in transparent specimen containers to accommodate their enlarged growth.

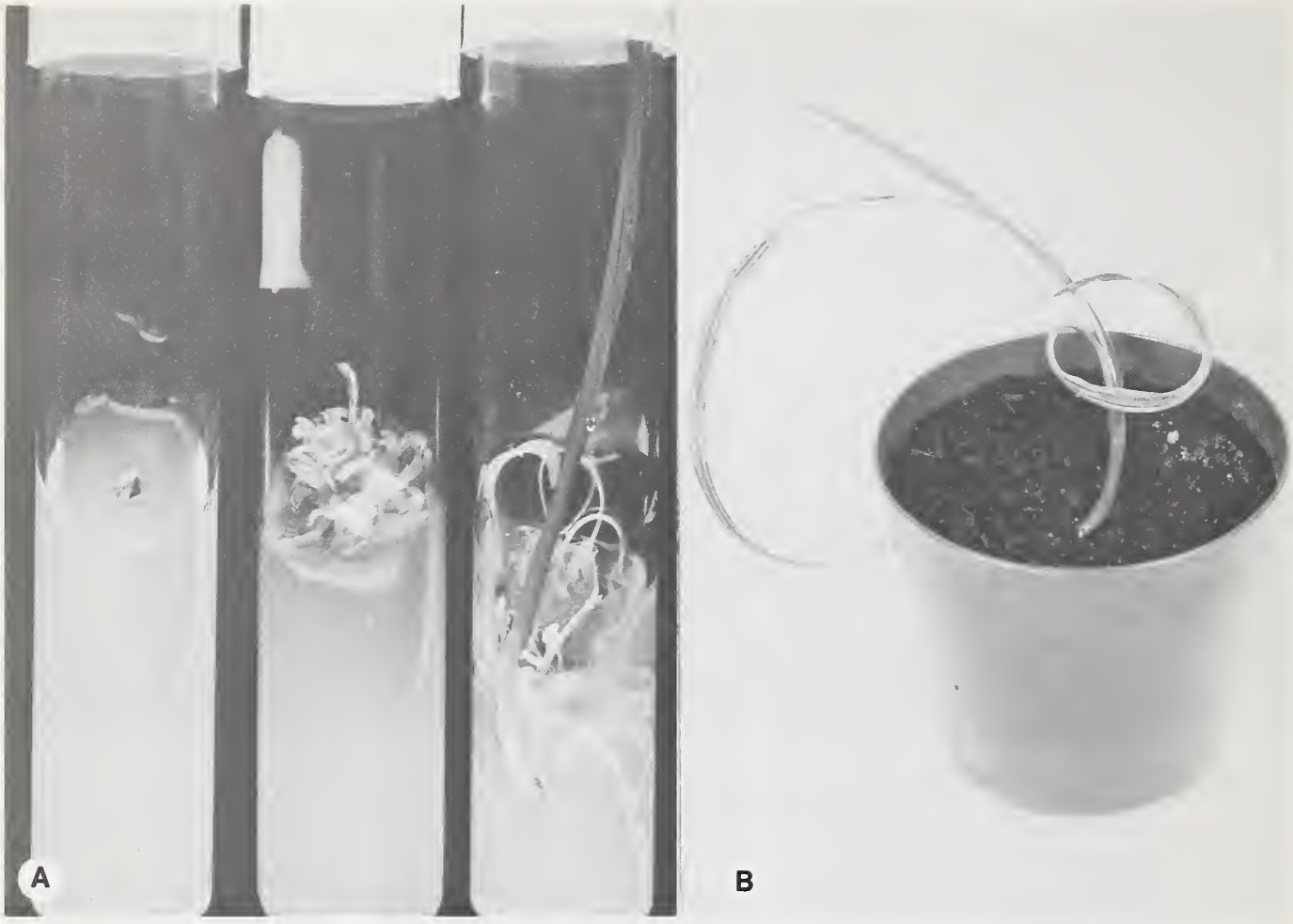


Figure 17.
Cloning date palms through shoot-tip culture: A (left), Initial shoot-tip, culture is about 2-wks old showing early leaf development; (middle), 6- to 8-wk old culture showing additional shoots from original shoot tip; (right), shoot tip producing adventitious root system, which may be successfully planted in soil. B, free-living plantlet derived from a shoot-tip explant that has been in soil for about 4 wks.

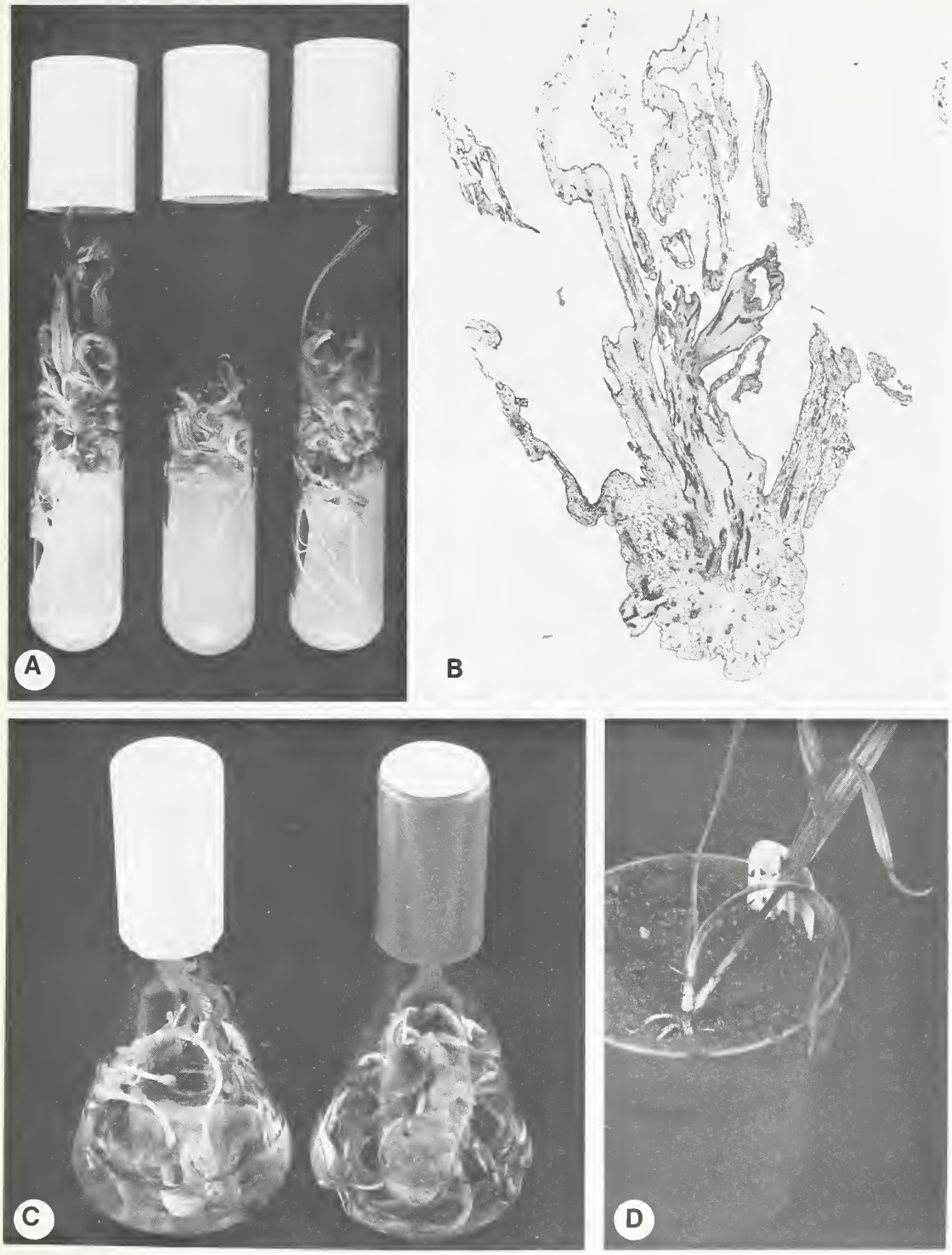


Figure 18.
Suckers from tissue culture derived date palm plantlets: A, Suckers produced from asexual plantlets derived from 'Medjool' callus; B, cross section of asexual plantlet with suckers; C, suckering on cultured zygotic embryo in vitro; D, sucker on an asexual plantlet derived from callus and recently transferred to soil.

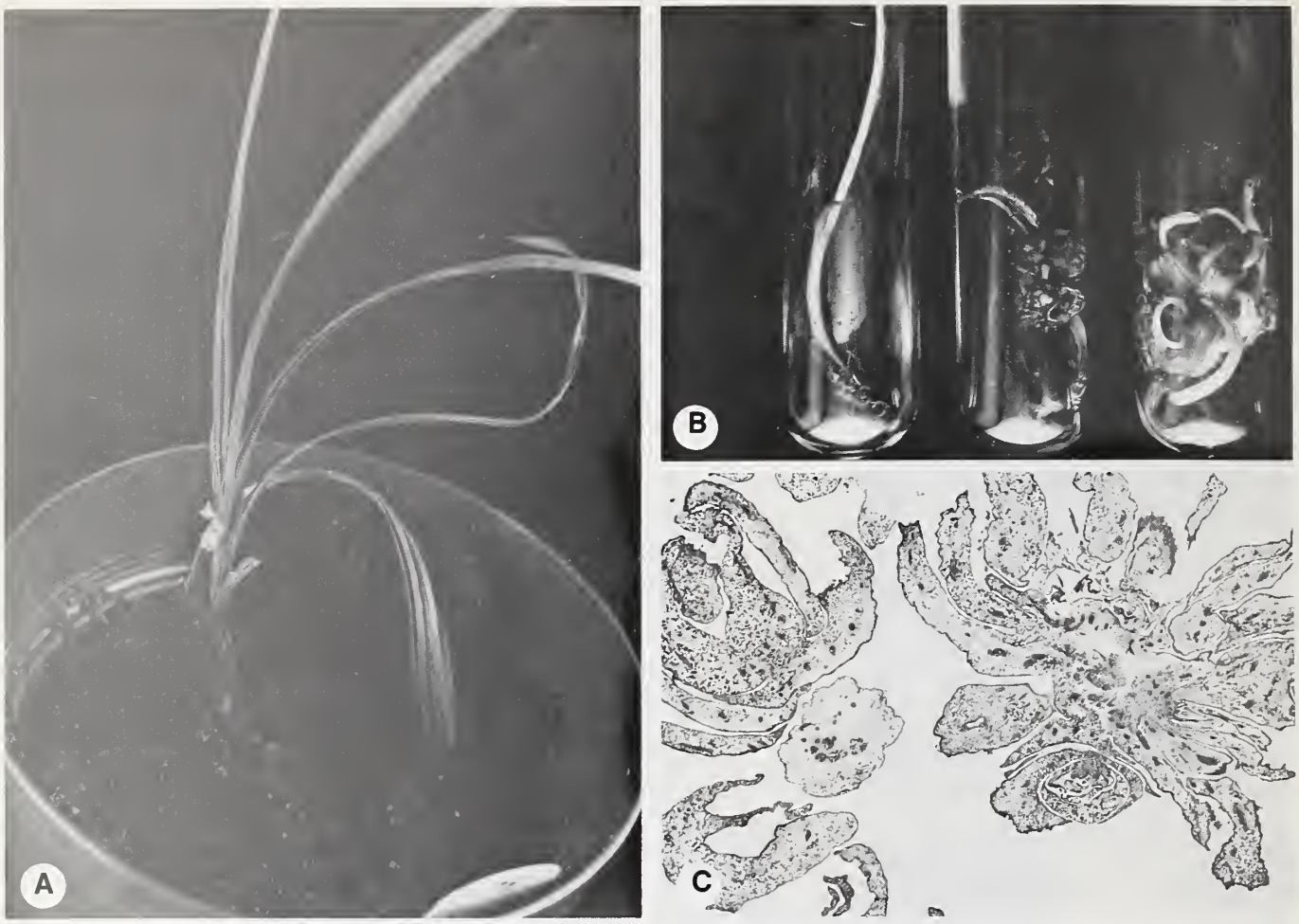


Figure 19.
Production of inflorescences from tissue culture derived date palm plantlets: A, 4-wk-old plantlet derived from an asexual embryo producing an inflorescence; B, various morphogenetic responses obtained from cultured zygotic embryos on nutrient medium containing 0.1 mg/L NAA and 10 mg/L BA, left to right, single-stemmed plantlet, middle, plantlet producing an inflorescence, and right, multiple-stemmed plantlet; C, cross section of a female inflorescence derived from a shoot-tip culture. Flower buds are compressed together unlike their natural occurrence.

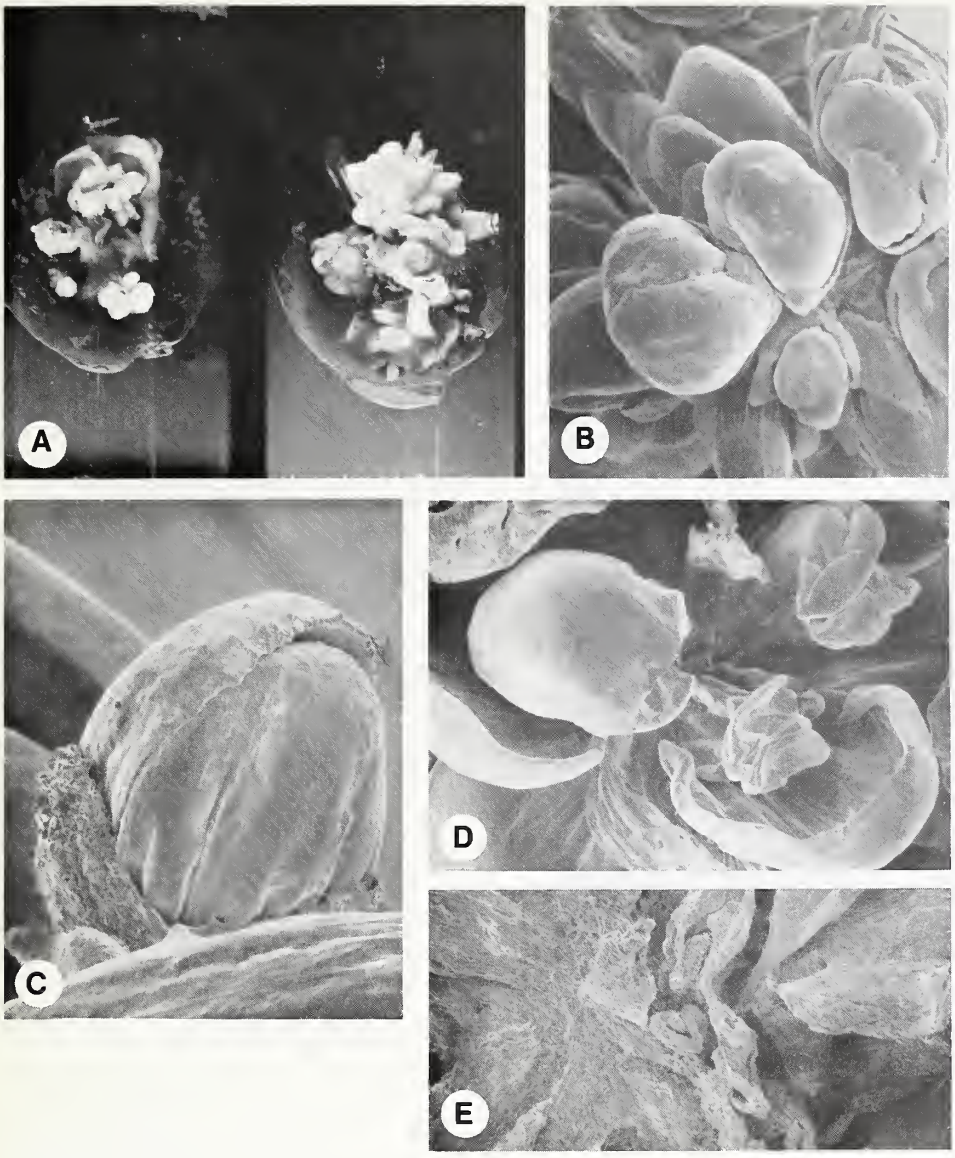


Figure 20.
Derived inflorescence produced in vitro from a date palm shoot-tip culture: A, shoot-tip cultures producing inflorescences--culture on the left is producing two inflorescences simultaneously; B, scanning electron microscopy examination of inflorescences reveals them to be composed of male flowers; C, magnified male flower bud; D, dissected male flower bud shows petals (outwardmost whorl), sepals (next inner whorl), and anthers (inner-most whorl); E, removing outside appendages reveals occurrence of pseudocarpels (centrally located protrusions).

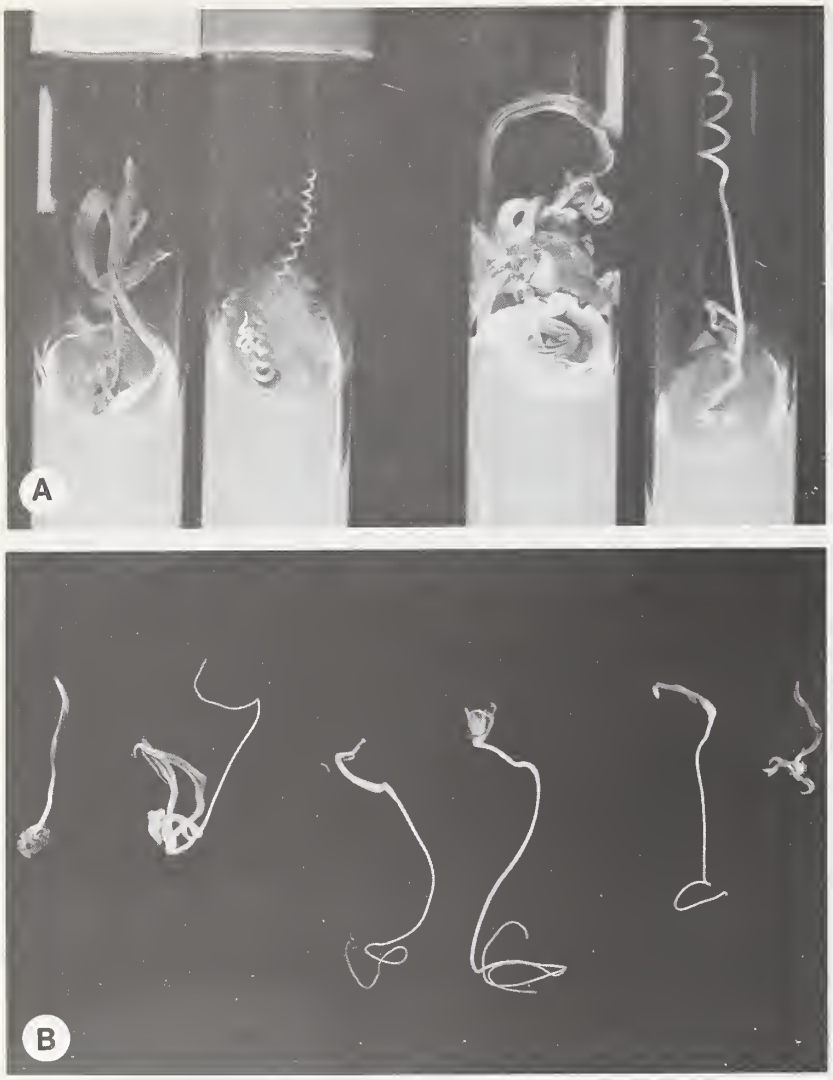


Figure 21.
Abnormalities occurring among plantlets derived from tissue culture techniques: A, curling observed in tissue culture of palm cultures on nutrient medium containing 0.1 mg/L NAA, normal shoot-tip development (left), abnormal shoot-tip development (left-middle), normal seedling development (right-middle), and abnormal seedling development (right). Cultures are about 16-wks old. B, examples of retarded or abnormal germination phenomena of plantlets derived from date palm callus cultures on basal medium containing 0.3 percent charcoal (left to right): Plantlet without primary root development, abnormal geotrophic responses (both root and shoot grow aerially), inhibited foliar leave emergence, multiple leaves emerging from slit-shaped opening, normal plantlet development, and plantlet without primary root development.

APPENDIX

List of Chemicals Mentioned

Common name	Chemical name
ascorbic acid	3-oxo-L-gulofuranolacetone.
benomyl	[1-[(butylamino)carbonyl]-1 H-benzimidazol-2-yl] carbamic acid methyl ester.
citric acid	2-hydroxy-1,2,3-propanetricarboxylic acid.
meso-inositol dihydrate	hexahydroxycyclohexane dihydrate.
naphthaleneacetic acid (NAA)	α-naphthaleneacetic acid.
sodium hypochlorite	same.
sucrose	B-D-fructofuranosyl-a-D-glucopyranoside.
2iP	N^6-(^{2}-isopentyl) adenine.
2,4-D	2,4-dichlorophenoxyacetic acid.
thiamine-HCl	3-[(4-amino-2-methyl-5-pyrimidinyl) methyl]-5-(2-hydroxylethyl)-4-methylthiazolium chloride monohydrochloride.
Tween-20	polyoxyethylene sorbitan monolaurate.

Glossary

adventitious. A structure arising from an unusual place: for example, buds produced at places other than from leaf axils and roots growing from stem or leaves.

agar. A solidifying agent used in nutrient media preparations and obtained from certain types of red algae (Rhodophyta).

anther. The reproductive upper portion of the stamen containing the pollen grains.

aseptic. A sanitary and sterile situation.

asexual embryogenesis. The sequential series of events whereby an embryo arises from somatic tissue and analagous to the development of a germinating sexual embryo.

auxins. Natural or artificial types of phytohormones characterized as substances that promote cell elongation (that is, IAA, IBA, NAA, p-CPA, and 2,4-D). In tissue culture, these substances are often associated with callus and root production.

axillary bud. A bud found at the axil of the leaf (synonymous with lateral bud).

callus. Dividing parenchymalike cellular tissue devoid of macroscopic organized structures.

carbohydrates. Organic compounds composed of carbon, hydrogen, and oxygen; for example, sugars and starches.

cell. Structural unit of living organisms; usually a plant cell consists of protoplasm surrounded by a cell wall in plants.

cotyledon. The initial leaf of the embryo.

complex addenda. Chemically undefined compounds added to nutrient media to stimulate growth.

culture. The living tissue grown in vitro within a single culture vessel.

cytokinins. Natural or artificial types of phytohormones characterized as substances that promote cell division (that is, BA, kinetin, and 2iP). In tissue culture, these substances are associated with enhanced callus and shoot development.

dioecious. Male and female flowers produced on separate plants.

development. Changes in the form in the plant body caused by differentiation and growth.

differentiation. Qualitative changes and differences that appear among cells, tissues, and organs during growth.

embryo. Initial plant form before germination.

embryogenesis. Process of embryo initiation and development to an autotrophic plant.

explant. Original part of the parent plant tissue that is introduced to in vitro conditions.

free-living conditions. Natural or greenhouse conditions where the plantlets are removed from in vitro conditions and transferred to soil mixtures. These plantlets must manufacture their own food supply to survive.

genetic variation. Occurrence of abnormal plantlets or cultures resulting from a genetic change during the tissue culture process.

germination. Developmental stages associated with production of a plant from an embryo.

growth. All quantitative changes during the life of an organism.

habit. General plant physical appearance.

habitat. Particular kind of environment in which plants live.

haustorium. An absorbing organ, in date palms the organ is appressed to the endosperm and is part of the cotyledon.

inflorescence. In palms, flower cluster or a spadix.

inorganic salts. Mineral nutrients necessary for survival of biological organisms.

in vitro. A sterile, artificial environment, typically in glass vessels, in which cultured cells, tissues, organs, or whole plants may reside.

lateral bud. Axillary bud produced at the base of a leaf petiole.

meristele. The vascular cylinder tissue in the stem.

micropropagation. Rapid vegetative propagation of a plant using tissue culture technology and usually beyond that obtainable in nature.

microsporangium. The structure in the anther that produces the pollen grains.

monocotyledon. A flowering plant with one cotyledon in the embryo.

morphogenesis. Study of the origin of form.

nutrient medium. A chemical formulation in which cells, tissues, and organs are grown in vitro; it provides nourishment and regulates morphogenetic events. Usually contains a source of inorganic salts, vitamins, carbohydrates, phytohormones, and sometimes, complex addenda.

organ. A tissue or group of tissues that constitute a morphologically and functionally distinct part of the organism (that is, leaf, inflorescence, root).

organogenesis. Initiation of an organ structure, or the production of a plantlet in vitro through sequential nonsynchronized initiation of root and shoot structures connected by vascular tissue.

phytohormones. Plant hormones that include auxins, abscisic acid, cytokinins, gibberellins, ethylene, and other related substances. Phytohormones are chemical messengers that may pass through cells, tissues, and organs and cause biochemical, physiological and morphological responses in the area of origin or remote from the area of origin.

plantlet. A plant with a distinct root and shoot system developed through tissue culture propagation either by embryogenesis or organogenesis.

pollen grains. Male gametophyte or microspore of the seed plants.

pseudocarpels. Vestigial-leaflike organs bearing ovules in male palm flowers.

reculture. Transfer of the whole culture, cell, tissue, organ, or plantlet intact from one medium to another.

shoot tip. Terminal bud of the plant; consists of the apical meristem and the immediate surrounding leaf primordia.

subculture. Division and transfer of a part of the culture to fresh medium.

suckering. Type of vegetative propagation where lateral buds grow out to produce an individual that is a clone of the parent.

tissue. A group of cells of similar structure that performs a special function.

tissue culture. A general term used to describe the development of plant cells, tissues, and organs cultured on nutrient medium in sterile conditions.

vitamins. Substances involved in the synthesis of coenzymes. These substances should be added to tissue culture preparations because they usually cannot be produced in vitro.